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CHARACTERIZATION OF PURINE RESISTANT MUTANTS IN *DROSOPHILA MELANOGASTER*

by

© SUDHA RAVISHANKER

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SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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To my parents and Ravi

ABSTRACT

Wild type strains of *Drosophila melanogaster* , specifically those with xanthine dehydrogenase activity (XDH^+), are resistant to moderate levels of purine added to their diet. In contrast, *rosy* flies (XDH^-) do not develop on such media, and hence are relatively purine sensitive. Purine resistant strains were selected by subjecting *rosy* (XDH^-) strains to EMS mutagenesis, and scoring for increased survival on purine-containing media. Seven purine resistant mutants were thus isolated, and when tested for XDH activity, were found to be XDH^-. This, in itself, suggested that a locus other than the *ry* locus was involved in conferring purine resistance to these mutants. In an attempt to identify the lesion(s), the seven purine resistant mutants were characterized genetically and biochemically.

The genes conferring purine resistance (Pur^R) were found to be recessive in all but one of the mutants, in which it is semi-dominant. In two of the strains (P.R. 51 and P.R. 130) the Pur^R mutant was found to map to chromosome 3, in one of them (P.R. 133) to chromosome 2 and in the other four mutants, the linkage data were not clear enough to make a definite chromosome assignment.

It has previously been reported by Johnson and Friedman (1981, 1983) that purine resistant mutants in *Drosophila* are deficient for adenine phosphoribosyl transferase activity (APRT) and that purine resistance maps to the *Aprt* locus, (map position 3:3.03). Thus the seven purine resistant mutants were assayed for APRT activity.

Three of the seven mutants are deficient for APRT activity ($APRT^-$). The other four, however, have varying levels of APRT activity compared to the wild type OR^R. One of them, P.R. 133, is particularly interesting as it has APRT levels substantially higher than wild type. This shows that a complete lack of APRT activity is not required to render flies resistant to purine.

In order to determine the number of loci involved in purine resistance, classical complementation analyses for the purine resistance phenotype and APRT activity were performed. Two of the mutants characterized by Johnson and Friedman, #82 and #172, were included as controls. The analyses indicated that at least one other locus besides those previously implicated, is involved in purine resistance. Although this locus has not been identified, some speculations have been made.

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TABLE OF CONTENTS

	PAGE
ABSTRACT	v
ACKNOWLEDGEMENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
I. INTRODUCTION	1
II. MATERIALS AND METHODS	13
Drosophila melanogaster stocks	13
Mutagenesis	13
Purine resistance	16
Axenic culture	17
Genetic characterization	17
Test for dominance of Pur^R	17
Linkage Analyses	18
Complementation Analyses: For purine resistance	18
Biochemical characterization	19
Assay for APRT	19
Thin layer chromatography	26
Fluorography, Autoradiography and quantitation of APRT activity	26
Protein determination	27
Complementation Analyses: for APRT activity	28

III. RESULTS AND DISCUSSION 29

Mutagenesis 29

Levels of Purine Resistance 30

Genetic characterization 35

Linkage analysis 38

Complementation analysis: for purine resistance phenotype 43

Biochemical characterization 48

APRT assays 48

Complementation analyses: for APRT activity 61

Summary 71

CONCLUSIONS 74

BIBLIOGRAPHY 78

APPENDIX 85

LIST OF TABLES

TABLE	DESCRIPTION	PAGE
1	Description of stocks.....	14
2(a)	Tests for dominance of the purine resistant phenotype	36
2(b)	Purine resistance phenotype in a ry^+ background	39
3	Tests for X-chromosome linkage of Pur^R.....	40
4	Tests of 2nd chromosome linkage of Pur^R.....	41
5	Tests for 3rd chromosome linkage of Pur^R.....	42
6	Complementation analyses: for purine resistance	46
7	APRT activities of the purine resistant mutants, and *ry* strains expressed as a percentage of wild-type activity	60
8	APRT activities in larvae	62
9(a)	Complementation results of APRT nulls	64
9(b)	APRT activity levels in the F_1's of P.R.$_R$ strains crossed with each other, and OR^R.....	66
9(c)	APRT activity levels in the F_1's of purine resistant, $APRT^+$ strains	69

LIST OF FIGURES

FIGURE		PAGE
1	Crosses to determine X-chromosome linkage of Pur^R	21
2	Crosses to determine 2nd chromosome linkage of Pur^R	23
3	Crosses to determine 3rd chromosome linkage of Pur^R	25
4(a)	Levels of purine resistance	32
4(b)	Levels of purine resistance	33
4(c)	Levels of purine resistance	34
5(a)	Autoradiograms of APRT assays	50
5(b)	Autoradiograms of APRT assays	51
5(c)	Autoradiograms of APRT assays	52
5(d)	Autoradiograms of APRT assays	53
6	Kinetics of APRT activity in OR^R	56
7	Kinetics of APRT activity in the purine resistant mutants, and wild-type and *ry* controls	58

I INTRODUCTION

As well as being components of ribo-and deoxyribonucleic acids, purines and their derivatives are involved in a large number of chemical reactions. They serve as substrates, cofactors and regulatory molecules in several metabolic pathways. Their physiological functions include mediation of hormone effects, platelet aggregation and vasodilation. Pharmacologically, they are useful as immunosuppressants, diuretics and inhibitors of viral and tumor growth (Henderson, 1972). A number of human diseases result from abnormalities of purine metabolism. For example, the Lesch-Nyhan syndrome results from deficiency of the purine 'salvage pathway' enzyme, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) (Seegmiller *et al.*, 1967). It is an X-linked recessive disease characterized by severe neurologic disorder, and pronounced overproduction of uric acid. A more common human disease associated with abnormality of purine metabolism is gout. One of the causes of gout is glucose-6-phosphatase deficiency (Howell *et al.*, 1962). Lack of this enzyme increases the concentration of endogenous phosphoribosyl pyrophosphate (PRPP) and this leads to an accelerated rate of purine biosynthesis *de novo*. In 1972, the first inborn error of adenosine metabolism was discovered in man. A deficiency of adenosine deaminase was found to be associated with severe immune dysfunction (Ullman *et al.*, 1976). Clearly, the study of purine biosynthesis and metabolism is of biological, biochemical, genetic and medical importance. These studies have been done in several prokaryotic and eukaryotic experimental organisms,

and it is likely that the information gained from those studies can be extended to humans.

The study of purine metabolism began as early as 1776, when naturally occuring purines and their derivatives were characterized (Henderson, 1972). Purines are found in cells predominantly in the form of nucleotides. Purine nucleotides can be synthesized *de novo* from non-purine precursors (reviewed in Henderson, 1972). Initial evidence for the occurrence of *de novo* biosynthesis of purines was indicated from the growth and normal development of mice on low purine diets (Socin, 1891). During the period between 1887 and 1959, the reactions involved in purine biosynthesis and metabolism and the enzymes catalysing these reactions were identified and characterized, mainly using pigeon and chicken liver. (This work has been reviewed in Buchanan, 1959; Hartman, 1970; Henderson, 1972). During the period 1946-1951, the precursors of the purine ring were identified using labelled compounds (Henderson, 1972). Currently, the focus of attention is the mechanism of regulation of purine biosynthesis. Broadly speaking, this involves a study of the physical and kinetic properties of the different enzymes involved in purine metabolism; the effect of substrate concentrations on the rate of the biochemical pathways; inhibition of the metabolic pathway by end-products ("feed-back inhibition"), and the effects of drugs and analogues on the biosynthesis of purines (Henderson, 1972).

Initial experiments to study purine biosynthesis and metabolism were nutritional studies, where purines or their derivatives were included in the growth media, and the effects of these on the biology

of the organism were noted. These studies were done in various systems: bacteria, viruses, cultured animal cells and in invertebrates including *Drosophila*.

During the course of nutritional studies in *Drosophila* it was found that certain purines stimulate growth or development when included in the culture media (House, 1962; Sang, 1978). For example, it was first shown by Schultz *et al.*, (1946) that dietary RNA improves larval growth. However, this was not surprising, as *Drosophila* is adapated to a yeast diet, using the nucleic acids available there. Whole DNA and RNA molecules were initially tested. DNA did not improve larval growth (Hinton, 1956). The constituents of RNA, particularly the purine bases and nucleosides are growth-promoting (Sang, 1978). Thus, adenine can stimulate growth of *Drosophila* larvae when included in culture media (Hinton, *et al.*, 1951; Hinton, 1956). Further, it was found that cultured cells in which the pathway for purine biosynthesis is blocked with methotrexate, can grow in the presence of adenine (Wyss, 1977). These findings were evidence for the utilization of purine bases by *Drosophila via* a "salvage pathway".

Although *de novo* pathways are the major routes by which purine and pyrimidine nucleotides are synthesized, the salvage pathways are alternative routes by which nucleotides are synthesized from free purine or pyrimidine bases and their derivatives. The purine bases can react with phosphoribosyl pyrophosphate (PRPP) to form the corresponding nucleotide. Thus the enzyme adenine phosphoribosyl transferase (APRT) catalyses the direct synthesis of adenosine

monophosphate from adenine and PRPP. Becker (1974) showed that cultured *Drosophila* embryo cell lines and extracts of adult flies had significant APRT activity. The enzyme is also implicated in adenine transport (Becker, 1974), as no labelled adenine was detected in cultured *Drosophila* cells deficient in APRT.

On the other hand, hypoxanthine-guanine phosphoribosyl transferase (HGPRT), an enzyme found in many organisms and which utilizes hypoxanthine and guanine as substrates, was not detected in any of the cultured *Drosophila* cell lines or in extracts of adult flies by Becker (*op. cit*). In a later study however, Becker (1978) reported that HGPRT activity was detectable if cultured cells were grown in the presence of certain purines, pyrimidines, antimetabolites or glutamine. Enzyme activity was also detected if the extracts from adult flies were dialyzed before the assay. Johnson *et al.* (1980 a, b) reported the utilization of dietary ^{14}C hypoxanthine and guanine *via* HGPRT in *Drosophila* larvae. However, the incorporation of these bases into nucleic acids was low. Examples of *Drosophila* mutants, as well as microbial and animal cell line mutants, deficient in phosphoribosyl transferases, are cited below.

In addition to the information gained from nutritional studies, mutational studies have greatly contributed to the elucidation of nucleotide metabolic pathways and their regulation. A geneticists' approach to studying biosynthetic processes is to isolate mutants affected in the genes governing these pathways. Thus, in microorganisms, auxotrophs have proved to be an important tool in the understanding of gene regulation and in deducing the sequences of

steps in a specific biochemical pathway. Auxotrophs are mutant in a gene encoding an enzyme of a given pathway leading to the synthesis of an essential compound. These mutants are therefore incapable of surviving on minimal medium, unless provided with a nutrient not required by the wild type.

Purine auxotrophs have been isolated in various microorganisms (see Nash and Henderson, 1982 for references). The first reported auxotroph in animals was an adenine requiring strain of *Drosophila* (Hinton *et al.*, 1951). A systematic search for nucleic acid auxotrophs in *Drosophila* was undertaken by Vyse and Nash, using ethylmethane sulphonate (EMS) mutagenesis to induce mutations. One mutant that they isolated (Vyse and Nash, 1969) had a dual requirement for purine and pyrimidine nucleosides (Vyse and Sang, 1971). Norby (1970) made an important discovery when he found that *rudimentary (r)* mutants are pyrimidine auxotrophs. Their biochemical defects were shown to involve one or more of the aspartate transcarbamylase (Norby, 1973), carbamyl phosphate synthetase (Jarry and Falk, 1974) and dihydroorotase (Rawls and Fristrom, 1975) activities. Further surveys produced about forty-five ribo-nucleic acid auxotrophs (Nash, personal communication). Of these, twenty mapped at, or close to, the *rudimentary* locus (Falk and Nash, 1974). Among the others, at least eleven are purine auxotrophs, and these map to seven loci. (For a summary of the information on purine auxotrophs in *Drosophila*, see Nash and Henderson, 1982). Although the structural genes associated with purine auxotrophy have not been identified, some speculations have been made. For example, the auxotroph *ade* 2-1

is believed to be defective in guanine deaminase (Nash and Henderson, 1982).

Another approach to the subject of nucleotide metabolism has been from studies on toxicity of nucleosides and nucleic-acid bases to fruit flies. While some purine bases and nucleosides are growth promoting, it was found that at higher concentrations, they have toxic effects. (For a summary of the literature on the effects of nucleosides and bases at various concentrations on wild type larvae, see El Kouni, 1977). A considerable amount of work has been done in the area of nucleoside and base toxicity, but controversies on the utilization and toxicity of these compounds can be found in the literature. For example, while Wilson (1942) found that adenine at a concentration of 2.0×10^{-3} M was lethal to wild type *Drosophila* larvae, Ville and Bissell (1949) found that a concentration of 4.0×10^{-3} was not lethal and, in fact, accelerated the rate of development of the larvae. These conflicts arose largely because of differences in strains and experimental designs used by the different workers (El Kouni and Nash, 1977). In order to circumvent this problem, El Kouni and Nash (*op. cit*) did a systematic study, using uniform techniques on wild type *Drosophila melanogaster* larvae. They tested the survival of the larvae on defined medium supplemented with various concentrations (ranging from 10^{-4} M to 3.16×10^{-2} M) of nucleic acid bases and nucleosides. They found that cytosine, thymine, thymidine and adenine, were completely lethal at the highest concentration tested ie. 3.16×10^{-2} M. Deoxyadenosine and

deoxyguanosine were lethal even at the concentration of 10^{-2} M. Further, they found that purine related compounds are generally more toxic than pyrimidine related ones.

The biochemical bases for the toxicity of purine bases and nucleosides is an important aspect of the study of purine biosynthesis and metabolism. The raw materials for these studies are mutant cell lines or strains (depending on the biological system under study), that are resistant to a particular purine or analogue. A common approach to study the mechanism of resistance, is to determine how the resistant strain differs biologically and biochemically from strains that are sensitive to the purine or analogue. By studying these mechanisms in experimental, multicellular organisms like *Drosophila*, it is likely that the information gained will increase our understanding of purine metabolism in man as well.

In the study of purine toxicity, one of the primary questions asked, is whether the administered purine base or nucleoside is itself the toxic agent or whether it has to be metabolized to a toxic form. For purine toxicity, it has often, though not always, been found that the purine base or nucleoside is converted to the nucleotide, which is the active, toxic form. (For a review on the mechanisms of toxicity of purine bases and ribonucleosides in animals and invertebrate cells, see Henderson and Scott, 1980).

An early example of such a study in microorganisms by Elion *et al.*, (1953), showed that a diaminopurine resistant strain of *Lactobacillus casei* failed to incorporate exogenous adenine into nucleic acids. Tomizawa and Aromow (1960) found that 6-mercaptopurine

(6-MP) resistance in mouse fibroblasts is due to an impaired capacity of these cells to form nucleotides from the free purine bases. Kalle and Gots (1961) found that resistance to purine analogues in mutants isolated from *Salmonella typhimurium* resulted from the loss of specific purine nucleotide phosphorylases. Studies by Taylor, *et al.* (1977) and Caskey and Kruh (1979) show that variants of cultured animal cells capable of growing in the presence of purine analogues, like diaminopurine and 8-azaguanine, are deficient respectively for APRT activity and HGPRT activity. One simple hypothesis for the mechanism of resistance is that the defective phosphoribosyl transferase can no longer convert the purine or analogues to a toxic nucleotide (Johnson and Friedman, 1981).

On the basis of the work done on bacterial cells and cultured animal cells, Johnson and Friedman (1981) tested purine resistant strains of *Drosophila melanogaster* for APRT activity and found that the strains lacked APRT activity. In a recent report (Johnson and Friedman, 1983), they show that purine resistance, deficiency for APRT activity and differences in the isoelectric points of APRT, result from alterations at a single locus, *Aprt* (map position 3:3.03).

Yet another approach to the study of purine metabolism in *Drosophila* was possible when certain eye color changes were found to result from alterations in xanthine dehydrogenase (XDH) activity. XDH is the principal enzyme of purine oxidation in *Drosophila* (Glassman *et al.*, 1968). It catalyses the conversion of hypoxanthine to xanthine, and of xanthine to uric acid in purine catabolism.

In pteridine metabolism, it converts 2-amino-4-hydroxy-pteridine to isoxanthopterin (Forrest *et al.*, 1956). Pteridines are purine derivatives (specifically guanosine triphosphate derivatives), that act as pigments in many insects including *Drosophila*. The conversion of 2-amino-4-hydroxy-pteridine to isoxanthopterin is the basis for a sensitive fluorimetric assay of XDH (Glassman, 1962).

Adult flies completely lacking XDH activity have dull reddish-brown eyes, in contrast to the bright-red of wild type eyes. Two mutants of *Drosophila* with a dull reddish-brown eye color were originally observed as reported in Bridges and Brehme (1944). One of these, called *rosy (ry)* mapped to chromosome 3, and the other, called *maroon-like (mal)* mapped to the X chromosome. Subsequently, several other mutations at the *ry* locus have been induced by X-irradiation and EMS mutagenesis (Chovnick *et al.*, 1978, Girton *et al.*, 1979). Other loci affecting XDH activity, *cin* (Baker, 1973) and *lxd* (Keller and Glassman, 1964) have also been described.

Glassman and Mitchell (1959) found that extracts of *ry* and *mal* mutants lack XDH activity. The eye color of XDH^- mutants does not result directly from the lack of isoxanthopterin, but from decreased amounts of orange-red pigments called "drosopterins". (Drosopterins are a group of dimeric pteridines). Isoxanthopterin is probably a precursor for these pteridines and this may be the reason for the dull reddish-brown eye color of XDH^- mutants. It is found that a modicum (.1%) of XDH activity is sufficient to restore wild type eye color (Gelbart *et al.*, 1976; Girton *et al.*, 1979). Phenocopies of *ry* or *mal* eye color can be produced by growing wild type flies

on media containing allopurinol (4-hydroxy-pyrazolo-(3,4-d)-pyrimidine), an inhibitor of XDH (Glassman, 1965). It has been reported that, in XDH^- strains, the larval malphigian tubules (which are the excretory organs in flies) become necrotic. This is thought to be due to an inability to dispose of, or to tolerate, hypoxanthine (Glassman, *op. cit.*). It is interesting to note that humans who lack xanthine oxidase (xanthinuria) are afflicted with kidney stones composed of xanthine and hypoxanthine (Avaizan, 1964).

Glassman (1965) found that mutants with reduced XDH activity failed to complete development on purine-containing media. The concentrations of purine that kill XDH^- larvae do not affect the development of wild type flies. This hypersensitivity of XDH^- larvae to purine has proven to be a very useful tool for the positive selection of purine resistant larvae, and has greatly facilitated fine structure mapping at the *ry* and *mal* loci. (See Glassman, 1965; Finnerty *et al.*, 1970; and Chovnick *et al.*, 1970, 1971 for some examples).

The curious feature about the toxicity of purine to developing cultures of XDH^- strains, is that purine is not a substrate for the enzyme, and the exact cause of death is still not known (reviewed in Nash and Henderson, 1982). A source of radiolabeled purine to do tracer studies might shed light on this problem.

Our laboratory was interested in isolating suppressors of nonsense mutations at the *rosy* locus. Hence, putative nonsense (CRM^-) *ry* mutants (Girton *et al.*, 1979) were subjected to EMS mutagenesis and the progeny selected for increased survival on purine containing

standard medium.

Among the purine resistant survivors, one expects to find at least three classes: first, the true revertants of the original mutation, which would have high levels of XDH activity, might occur; secondly, one might be able to isolate suppressors of the nonsense mutations at the *ry* locus, which would be identified by wild-type eyes, and probably, as having fairly low levels of XDH activity; thirdly, it may be possible to get mutants that have lesions at other loci involved in purine metabolism. For example, mutants at loci coding for permeases would reduce access of the purine to its target organs, or perhaps, mutants at yet other loci might result in a reduction of the toxicity of purine by blocking or modifying its metabolic fate.

Mutagenesis and selection of progeny on purine containing medium was carried out as described in Materials and Methods. A few revertants were isolated, which were recognized by wild-type levels of XDH activity. When the strains derived from the other purine resistant survivors were tested for XDH activity, it was found that they were XDH^-. In other words, suppressors of nonsense mutations at the *ry* locus had not been induced. Instead, we now had an interesting class of mutants that, presumably, fell into the last category of mutants mentioned above. They are XDH^- like *ry* flies, but purine resistant like ry^+ flies. A total of seven such purine resistant (hereafter referred to as P.R.) strains were isolated: five of them by John Bell, and two isolated by myself.

The purpose of the present study was to characterize the seven P.R. mutants obtained in our laboratory, both biochemically

and genetically. The biochemical basis for the resistance was one of the questions addressed, as it was hoped that this would shed some light on why purine kills developing cultures of XDH^- strains. Since Johnson and Friedman's work (1981, 1983) suggests that purine resistant strains of *Drosophila* are $APRT^-$, the seven P.R. strains isolated in this laboratory were assayed for APRT activity. It was found that three of the seven are complete nulls, while the other four have varying levels of APRT activity. One of them, P.R. 133 is particularly interesting, as it has activity substantially higher than wild type. Linkage analyses show that purine resistance maps to chromosome two in some mutants, and chromosome three in some others. Taken together with the differences in APRT activity, this shows that purine resistance in *Drosophila* may involve mutations at more than one locus.

II MATERIALS AND METHODS

A. *Drosophila melanogaster* stocks

All *Drosophila* stocks were routinely maintained on a standard yeast-sucrose medium (Nash and Bell, 1968) at room temperature (23 ± 2°C). A complete list of all *Drosophila* stocks used is given on Table 1. Oregon R was used as the wild type control in all experiments.

B. Mutagenesis

Purine resistant mutants were obtained by subjecting purine sensitive *ry* strains to ethyl methane sulphonate (EMS) mutagenesis. The procedure used was that of Lewis and Bacher (1968). Young males (0-48 hours old) were collected and transferred to bottles containing filter paper circles saturated with a solution of 0.015 M EMS in sterile 1% sucrose. The flies were allowed to feed for 18-24 hours, after which they were transferred to new bottles containing standard medium. The bottles and glassware used for mutagenesis, were decontaminated by rinsing with a solution of 0.5% thioglycolate in 1N NaOH.

The mutagenized males were mated to virgin females of the same strain and placed on standard medium. The F_1's were transferred to fresh bottles, allowed to mate, and the resulting F_2's were left to lay eggs in fresh bottles for two days. The parents were then discarded and 1.0 ml of 0.2% aqueous purine (Sigma) was added to the bottles. This level of purine is normally lethal to developing cultures of *ry* strains, and will therefore, permit the recovery of purine resistant strains.

Table 1: Description of Stocks

	Stock	Brief Description	Source
1a.	OR^R	Oregon R, wild-type	John Bell University of Alberta (U of A) Edmonton, Alberta
1b.	Amh	Amherst, wild-type	
	PURINE SENSITIVE STOCKS		
2.	*b cn; e* ry^{604}	Dark body, orange eyes	John Bell U of A
3.	*b cn;* ry^{8}	Body darker than wild type, orange eyes	John Bell U of A
	PURINE RESISTANT (P.R.) STOCKS		
4.	P.R. 51	Obtained by mutagenizing the purine sensitive *b cn; e* ry^{604}	John Bell U of A
5.	P.R. 94		
6.	P.R. 130		
7.	P.R. 131		
8.	P.R. 133		
9.	P.R. 8-1	Obtained by mutagenizing the purine sensitive *b cn;* ry^{8} stock	This study
10.	P.R. 8-11		
11.	#82	Purine resistant, APRT and is in a *ry* background	Victoria Finnerty Emory University Georgia
12.	#172	Purine resistant, APRT and is in a *mal* background	Victoria Finnerty Emory University

STOCKS USED FOR
LINKAGE ANALYSIS

13.	*SM5 Cy/Sp; ry*	SM5: Complete balancer for chromosome 2. Curly wings; Purine Sensitive	John Bell U of A
14.	*Tp(3) MKRS M(3) S(34) kar ry^{2} Sb/ kar^{2} Df(3) ry^{75}*	*Balancer for chromosome 3. *kar* and *ry* mutations together produce an orange eye color. Stubble bristles; Purine Sensitive.	John Bell U of A
15.	*$\widehat{XX}$:cn; ry*	Females have yellow bodies, forked bristles and orange eyes. Males have wild type bodies and bristles, and orange eyes; Purine Sensitive.	John Bell U of A

*This stock is referred to as the *MKRS* stock in the text.

Seven purine resistant strains were isolated. These were rechecked for purine resistance and then maintained on standard medium. On alternate generations, they were reselected on purine containing medium. This will ensure continued presence of the genetic factors responsible for purine resistance.

C. Purine Resistance

As mentioned above, the purine resistant strains were initially isolated on 0.2% aqueous purine. In order to determine the levels of purine resistance in the different strains, the larvae were allowed to develop on media containing various concentrations of aqueous purine.

Adult flies from the different purine resistant stocks, OR^R (the wild type control) and a *ry* strain, (ry^{604}) were pre-fed on yeast-paste for a day to optimize egg yield. The flies were then transferred to clean, empty quarter-pint bottles. These bottles were capped with egg-laying dishes containing 1.5% agar. The flies were allowed to lay eggs for about 6 hours, after which the egg-laying dishes were collected and kept in the 25°C incubator until the eggs hatched (approximately 20-24 hours).

First instar larvae were collected from the dishes, using the tip of a scalpel to pick up the larvae. One hundred larvae were placed in each vial. Two hundred microlitres (0.2 ml) of various concentrations of aqueous purine were added to the vials. The concentrations of purine ranged from 0.05% to 1.5%. Vials to which 0.2 ml of sterile water was added and vials to

which no supplement was added, served as zero-purine controls.

The number of flies surviving the different concentrations of purine was scored.

D. Axenic Culture

The purine resistant strains, and the stocks to which they were crossed, were grown and maintained under sterile conditions for some of the experiments. The adult flies were fed on yeast-paste for 1-2 days, and then transferred to clean quarter-pint bottles capped with egg-laying dishes containing 1.5% agar medium. The egg-laying dishes were collected after no more than 12 hours. The eggs were sterilized by immersion for 20 minutes in a solution of filtered, saturated calcium hypochlorite. Eggs were then filtered and rinsed in sterile Ringer's solution five times. The filter paper, containing the sterilized and rinsed eggs, was cut into pieces and placed in sterile culture vials.

Before eclosion of the flies, the medium was streaked out on microbial culture medium to check for sterility. This was also done periodically to ascertain whether the stocks were still axenic.

E. Genetic Characterization

1. To determine if the mutations that confer purine resistance are dominant or recessive, each of the purine resistant stocks was crossed reciprocally to the parent purine sensitive stock from which it was derived. The crosses were done in vials. The flies were brooded twice:

to one brood 0.2 ml of 0.2% purine was added, and to the other brood, which served as a control, no purine was added.

Similar crosses to OR^R (ry^+) were carried out to ascertain whether dominance was modified by the presence of *ry*+in the genetic background.

2. Linkage Analysis

In order to determine whether purine resistance maps to the X, second, or third chromosome, the crosses outlined in Figures 1, 2 and 3 respectively, were performed. To implicate second and third chromosome linkage, the segregation pattern of the purine resistance phenotype from the dominant markers *Cy* and *Sb*, respectively, was noted. A scheme using an attached X-chromosome was carried out to test for X-linkage.

3. Complementation Analysis: For Purine Resistance Phenotype

In order to determine the number of loci involved in purine resistance, classical complementation tests were performed. Reciprocal crosses were made between the purine resistant stocks, and the F_1's were allowed to grow on media with and without purine. The F_1 survivors in the experimental and control vials were scored. If the number of survivors in the control vials substantially exceeded those in the experimental vials, it was inferred that complementation did occur. On the other hand, if approximately equal numbers of survivors were present in

the broods with and without purine, it was taken to indicate lack of complementation and to define the two mutant strains as containing allelic resistance mutations.

F. Biochemical Characterization

1. Assay for Adenine Phosphoribosyl Transferase (APRT)

The fly homogenates were prepared by a modification of the procedure reported by Johnson and Friedman (1981). Crude extracts of the flies were prepared by grinding them in an extraction buffer at $4^{o}C$ utilizing a glass tissue-homogenizer. The buffer contained 60 mM Tris-HCl (pH 7.5), 0.1 mM Na-EDTA, 26.0 mM $MgCl_2 \cdot 6\ H_2O$ and 2.0 mM dithiothreitol (DTT). The flies were at a concentration of 150 mg/ml buffer, using approximately equal numbers of males and females. The homogenate was centrifuged at 10,000 x g for 10 minutes. The portion of the supernatant below the lipid layer was used for the assays.

The assay mixture contained in addition to the extraction buffer, 6.25 mM Na-PRPP (Sigma), 7.6×10^{-5} M (8 - ^{14}C) Adenine (17.9 μ Ci/μ mole or 57 μ Ci/μ mole, Amersham) and 10 μl of the fly homogenate in a total volume of 35 μl. The remaining fly homogenate was saved for a protein determination.

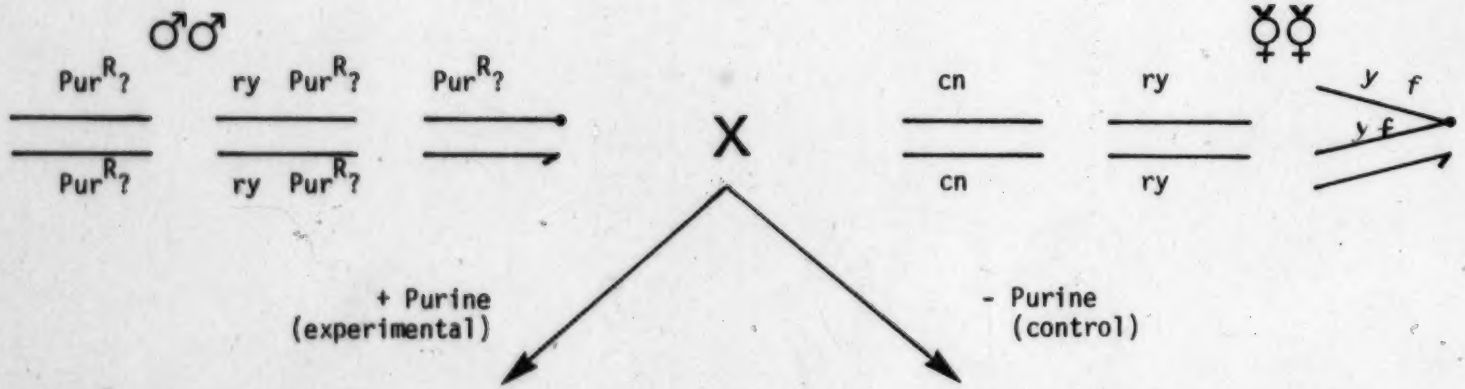
APRT catalyses the synthesis of adenosine monophosphate (AMP) from adenine and phosphoribosyl pyrophosphate (PRPP). APRT activity was measured at room temperature and is expressed as the mean counts per minute (cpm) of

Figure 1 X Chromosome Linkage Analysis of Pur^R

The purine resistant males were crossed to virgin females of the $\widehat{XX};cn;ry$ stock. The female progeny receive their X chromosomes from the mother, and the male progeny receive their X chromosomes from the father. The F_1's were selected on medium with (+P, experimental) and without (-P, control) purine. The experimental vials contained 0.2 ml of 0.2% aqueous purine.

NOTE: Pur^R refers to the gene that confers purine resistance.

Cross #1



If Pur^R resides on the X-chromosome, then the F_1 survivors will all be males.

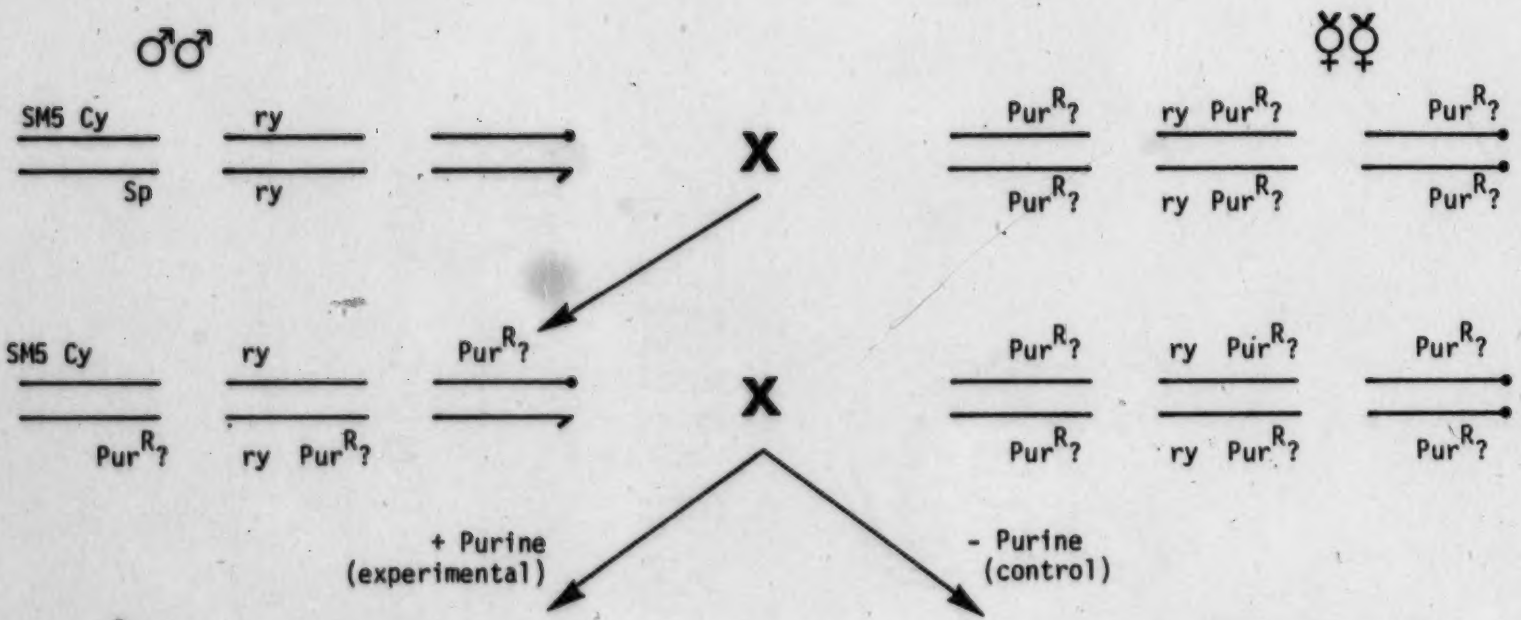
Male and female progeny should be found.

Figure 2 Second Chromosome Linkage Analysis of Pur^R

The purine resistant virgin females were crossed to SM5 *Cy/Sp;ry* males.

SM5 is a balancer for chromosome 2, and has the dominant markers *Cy* and *Sp* on it. The strain is also homozygous for *ry* on chromosome 3, thus making it purine sensitive. The F_1 *Cy ry* males were back-crossed to purine resistant virgin females, and the F_2's were selected on medium with (+P, experimental) and without purine (-P, control). The experimental vials contained 0.2 ml of 0.2% aqueous purine.

Cross #2



If Pur^R resides on chromosome 2, no (or very few) *Cy* survivors will be present. If a substantial number of *Cy* and Cy^+ flies are present, then the segregation of Pur^R is independent of chromosome 2, i.e.: Pur^R is not located on chromosome 2.

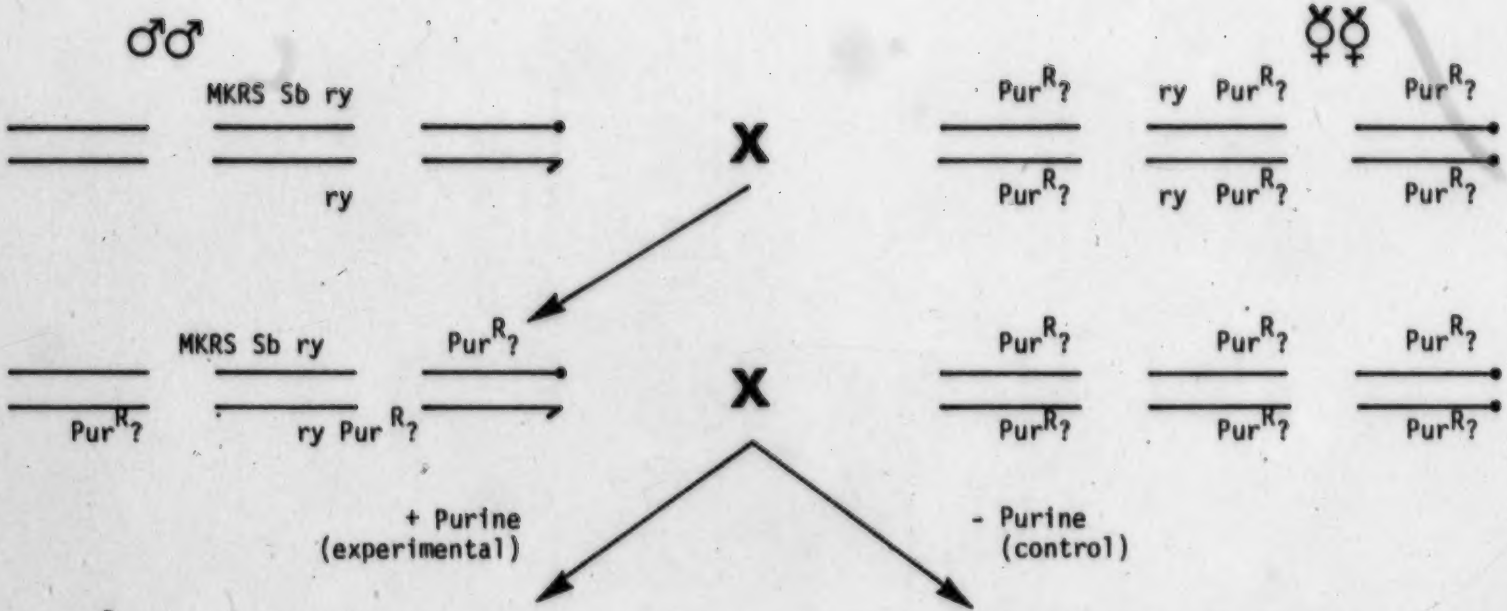
Approximately equal numbers of *Cy* and Cy^+ survivors should be present.

Figure 3. Third Chromosome Linkage Analysis of Pur^R

The purine resistant virgin females were crossed to males of the MKRS strain. This strain is a third chromosome balancer and has the dominant marker *Sb* on chromosome 3. It is homozygous for *ry*, and hence purine sensitive.

The F_1 *Sb ry* males were back-crossed to purine resistant virgin females. The F_2's were selected on medium with (+P) and without (-P) purine. The experimental vials contained 0.2 ml of 0.2% aqueous purine.

Cross #3



If Pur^R resides on chromosome 3, no (or very few) *Sb* survivors will be present. If a substantial number of *Sb* and Sb^+ flies are present, then the segregation of Pur^R is independent of chromosome 3, i.e.: Pur^R is not located on chromosome 3.

Approximately equal numbers of *Sb* and Sb^+ survivors should be present.

(8 - ^{14}C) AMP per microgram of protein.

2. Thin Layer Chromatography (TLC)

Poly (ethyleneimine) (PEI) - TLC plates (20 x 20 cms, purchased from Terochem) were used for the separation of the compounds.

Separate 2 µl aliquots of the reaction mixture were removed at 5, 10, 20 and 30 minutes after the initiation of the reaction. These were applied to the TLC plates, 2 cm from the bottom edge, at points previously overlaid with 2 µl of unlabelled 5' AMP and adenosine (both at 2 mg/ml). The plates were developed in 0.1 M Lithium chloride (Sigma), and dried. The positions of the AMP and adenosine markers were traced out using short wave ultraviolet light.

3. Fluorography, autoradiography, and determination of the radioactivity associated with AMP and adenosine

After the AMP and adenosine markers were traced out, the TLC plates were sprayed with Enhance (New England Nuclear). They were then exposed at -60°C for 2-3 days to X-ray film (Kodak), after which the films were developed.

The spots corresponding to AMP and adenosine were scraped off the TLC plates using a sharp one-edge razor blade. The radio-activity was quantitated by liquid scintillation counting.

At least four independent determinations were obtained for each of the purine resistant strains tested. The wild type OR^R was also assayed each time as a control.

In order to assay for APRT activity in larvae, the same procedure was used, except that 2^{nd} - 3^{rd} instar larvae were homogenized instead of adult flies.

4. Protein Determination

The Coomassie brilliant blue G assay for proteins described by Spector (1978) was used to quantitate the protein in the fly homogenates.

The dye reagent was prepared as follows:- One hundred mg of Coomassie brilliant blue G (Sigma) was dissolved in 50 ml of 95% ethanol. To this was added 100 ml of 85% (w/v) ortho phosphoric acid. The solution was diluted with water to a volume of 1 litre, followed by filtration. The final reagent was stored at room temperature, and was stable for two weeks. For an actual protein assay:- 2.5 ml of the dye reagent was added to 100 µl of the appropriate dilution of the fly extract, and the mixture vortexed. After letting the mixture stand for 5-10 minutes at room temperature, the absorbance at 595 nm was read on a spectrophotometer.

Bovine serum albumin (BSA) was used to generate a new standard curve for each set of assays. The blank contained 100 µl of water plus 2.5 ml of the dye reagent.

5. Complementation Analysis of APRT Activity

Complementation tests for APRT activity were carried out by making reciprocal crosses between the purine resistant strains, and assaying the F_1's for APRT activity. Besides our seven purine resistant mutants, two others, mutant #82, and #172, were also included in the complementation matrix. These two mutants were given to us by Victoria Finnerty, and were among the mutants characterized by Johnson and Friedman (1981, 1983). These mutants were found to be APRT nulls, and thus served as $APRT^-$ controls in the present experiment.

Each of the purine resistant strains were also crossed reciprocally to OR^R flies, and the F_1's assayed for APRT activity.

III RESULTS AND DISCUSSION

1. Mutagenesis

Purine sensitive *ry* males were subjected to EMS mutagenesis, and the mating schemes were carried out as described in the Materials and Methods. The hypersensitivity of developing *ry* cultures to purine was taken advantage of in selecting for purine resistant mutants. Among the survivors of the purine lethal screen, a few revertants were identified by the ry^+ eye color and high XDH activity as defined by the fluorometric assay for XDH (Girton *et al.*, 1979). None of the survivors with ry^+ eye color had low XDH activity, thereby effectively ruling out the possibility of a suppressor having been induced (see introduction). However, a group of purine resistant mutants were isolated that were, nonetheless, devoid of XDH activity. These mutants had a dull-red eye-color or an orange eye-color depending on whether they were isolated from a *ry* background (ry^{604}) or a *cn ry* background ($cn;ry^8$). Seven purine resistant mutants were isolated. Five of these, P.R. 51, P.R. 94, P.R. 130, P.R. 131, and P.R. 133 were isolated from ry^{604}, and the other two, P.R. 8-1 and P.R. 8-11 were isolated from *b cn; e* ry^8. These mutants were tested for XDH activity several times, and are XDH^- as larvae as well as adults. Thus these mutants are interesting in that they are XDH^- like *ry* flies but purine resistant like ry^+ flies. The fact that purine resistance is independent of XDH activity, in that both *ry* and ry^+ strains can be purine resistant, suggests that the genetic

situation at loci other than the *ry* locus is implicated in the purine sensitivity of *ry* strains. It was hoped that the genetic and biochemical characterization of these purine resistant mutants would reveal the nature of these loci.

2. Levels of Purine Resistance

The purine resistant mutants were selected at a concentration that kills developing cultures of *ry* strains (ie. 0.2% purine). Thus, although they are all more resistant than *ry* cultures at that purine concentration, it is of interest to measure the survival of these mutants on higher concentrations of purine. It is possible that some of the mutants may be as resistant or even more resistant than wild type. The wild-type strain, OR^R, and a purine sensitive strain, ry^{604}, were included as controls. Also, one of the purine resistant strains characterized by Johnson and Friedman (1981, 1983), #172 was included as an additional control. Figures 4a, 4b and 4c show the percent survival of the strains tested on the different concentrations of purine, at 25° C. It can be seen with 1% and 1.5% purine, strains P.R. 51, P.R. 94, P.R. 130, P.R. 131, P.R. 8-11 and #172 are even more resistant than the wild-type, although at some lower concentrations, all except #172 are significantly less resistant than wild-type. At 0.2% purine the survival of P.R. 133 is comparable to that of the other mutants, but at higher concentrations, there are no survivors. The survival of P.R. 8-1 on the different concentrations of purine is low. Since the stocks were

Figures 4a, 4b and 4c. Levels of Purine Resistance in the Purine Resistant Mutants, OR^R and ry^{604}.

A hundred first instar larvae of each strain were transferred to vials, to which different concentrations of aqueous purine were added. The number of survivors at the different concentrations was scored. The percentage survival is plotted against the purine concentrations. Each point on the graph represents an average of at least two determinations. The same values of OR^R and ry^{604} are plotted in Figures 4a, 4b and 4c.

Fig 4a.

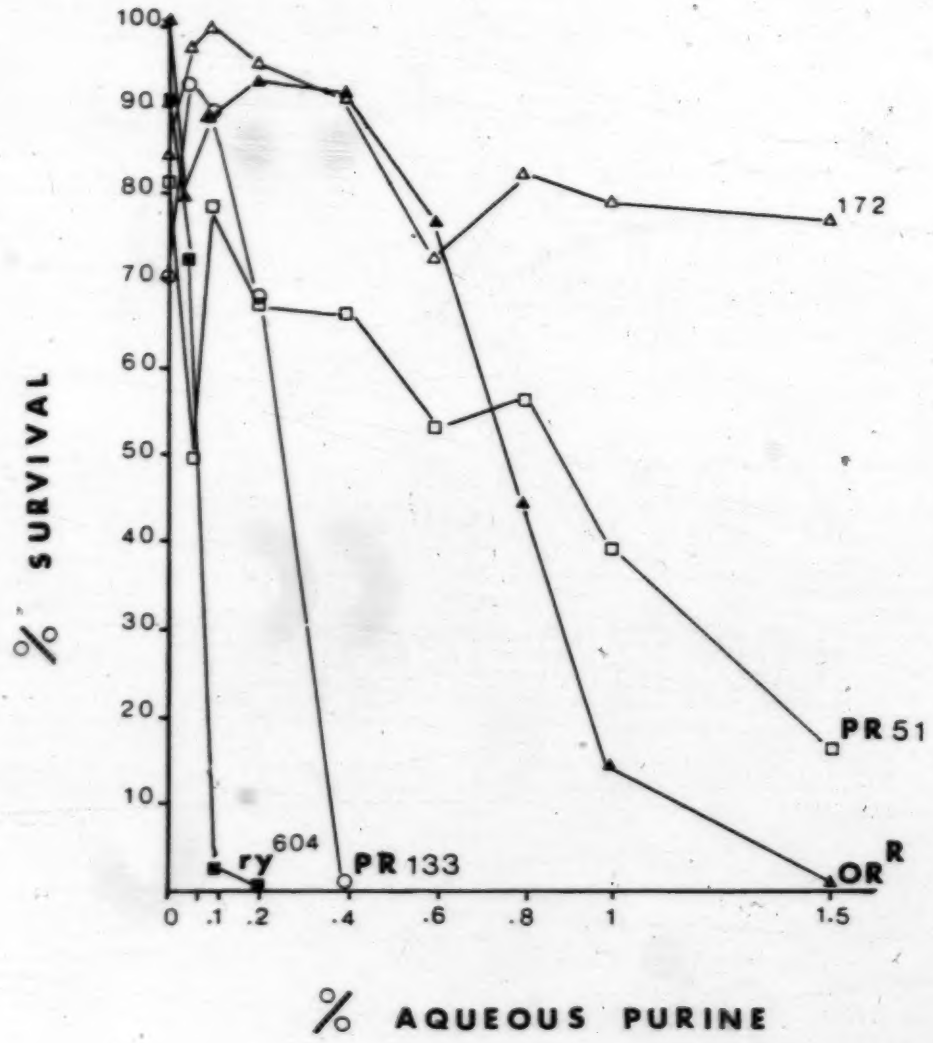

100
90
80
70
60
50
40
30
20
10
% SURVIVAL
172
PR 51
ry 604
PR 133
OR R
0
.1
.2
.4
.6
.8
1
1.5
% AQUEOUS PURINE

Fig 4b

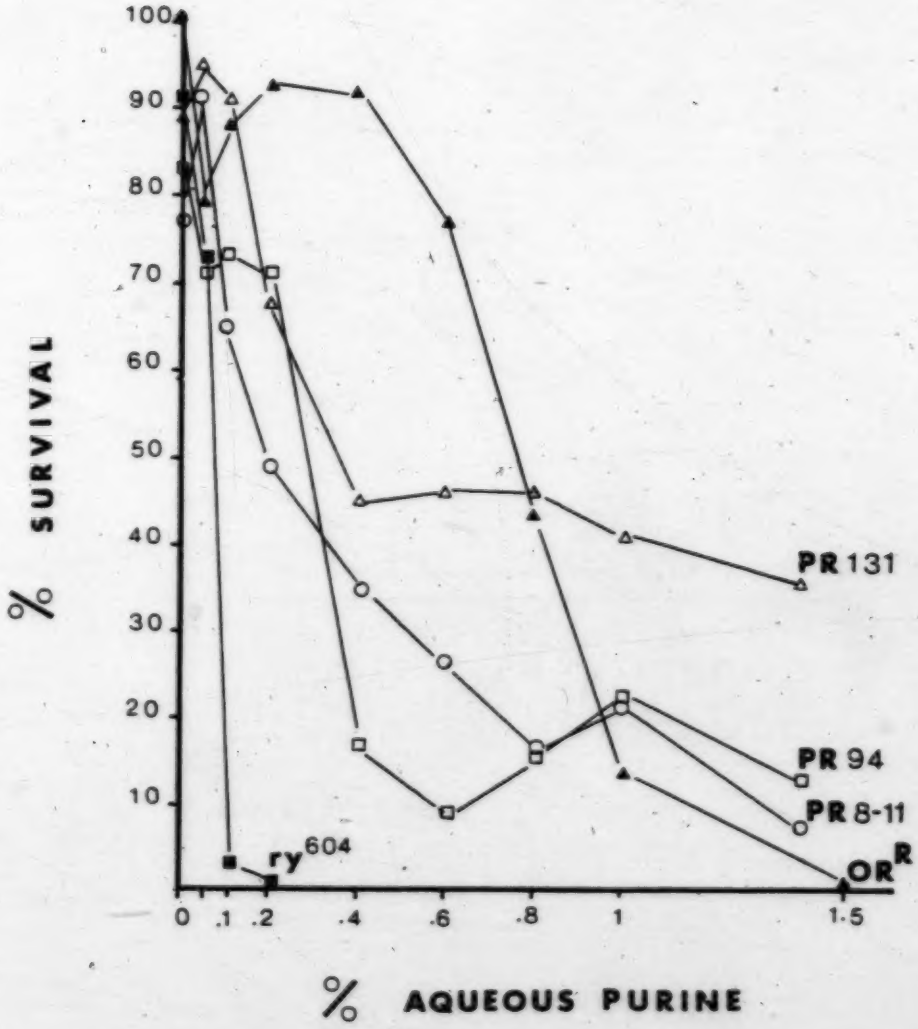
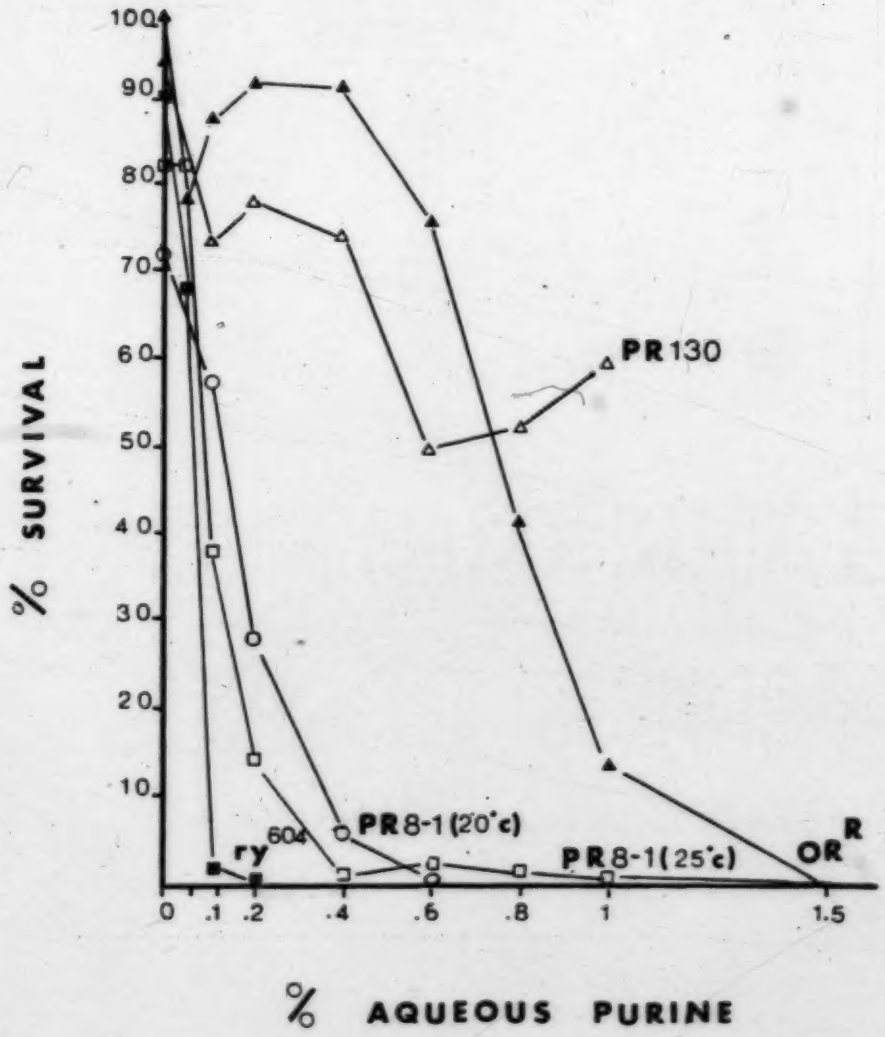

100
90
80
70
60
50
40
30
20
10
% SURVIVAL
PR 131
PR 94
PR 8-11
ry 604
OR R
0
.1
.2
.4
.6
.8
1
1.5
% AQUEOUS PURINE

Fig 4c


100
90
80
70
60
50
40
30
20
10
% SURVIVAL
PR130
PR8-1(20°c)
ry 604
PR8-1(25°c)
OR R
0
.1
.2
.4
.6
.8
1
1.5
% AQUEOUS PURINE

routinely maintained at room temperature and the present experiment was done at 25° C, it seemed pertinent to test if the low survival of 8-1 was due to temperature effects. Thus the larvae were allowed to develop on purine at a lower temperature, namely 20° C. As it can be seen from Figure 4c, there was no significant change in the survival profile of P.R. 8-1.

The number of survivors in vials to which sterile water was added and vials to which no supplement was added (controls), was not significantly different, thereby showing that mortality was not caused by drowning.

3. Genetic Characterization

In order to determine if the mutations conferring purine resistance are dominant or recessive, the purine resistant mutants were crossed reciprocally to the purine sensitive strains from which they were derived. The number of F_1 survivors on medium with and without purine was noted. Since no significant difference was found between the results of reciprocal crosses, the pooled data are presented in Table 2a. These results are from crosses done with axenic cultures. The crosses were also performed with non-axenic cultures, and the results were not found to be significantly different from those performed with axenic cultures. Thus, all the other experiments were done with non-axenic cultures.

The results presented in Table 2a compare the numbers of survivors in the experimental vials with the control vials.

Table 2 (a) Test for Dominance of Purine Resistance

Cross	Number of survivors in the experimental vials (+ Purine)	Number of survivors in the control vials (- Purine)	Conclusions
P.R. 51 x ry^{604}	40	394	Recessive ?
P.R. 94 x ry^{604}	52	559	Recessive ?
P.R. 130 x ry^{604}	69	273	Semi-Dominant ?
P.R. 131 x ry^{604}	52	583	Recessive ?
P.R. 133 x ry^{604}	0	562	Recessive
P.R. 8-1 x ry^{8}	38	458	Recessive ?
P.R. 8-11 x ry^{8}	2	189	Recessive ?
Control: ry^{604}	0	294	Purine Sensitive

The purine resistant mutants were crossed to the purine sensitive *ry* strains from which they were derived. The flies were brooded twice; to one brood, 0.2 ml of 0.2% purine was added, (experimental vials), and no purine was added to the other brood (control vials). The numbers of survivors in the experimental and control vials are compared.

The number of survivors of ry^{604} on medium with and without purine is also shown.

With one exception (P.R. 130), the numbers of survivors on purine containing medium are approximately equal to, or less than, 10% of the survivors in the control vials. The strain P.R. 133 is the only one that behaves as a complete recessive with respect to purine resistance. The strain P.R. 130 seems to behave as a semi-dominant with respect to purine resistance. In the other strains however, it is not absolutely clear whether purine resistance behaves as a recessive or a semi-dominant. There is a good correlation between the levels of 'leakiness' in the dominance tests and the survival on 1% purine (Figures 4a, 4b and 4c), implying that the effect is due to semi-dominance. But the results from the crosses involving the P.R. strains and the $\widehat{XX}$ strain, implies that purine resistance behaves as a recessive. Thus it is not possible to make definite conclusions regarding the nature of these mutations. Alternatively, it can be hypothesized that the presence of modifiers, accumulated since the isolation of these P.R. strains, could have produced the observed effects. This can be tested by out-crossing the purine resistant strains, and noting if the 'semi-dominant effect' is thus lost or not.

The purine sensitive ry^{604} strain was also allowed to develop on medium with and without purine. As can be seen from the results on Table 2a, there are no survivors in the experimental vials, thereby confirming that the strain is, indeed, purine sensitive.

In order to compare purine resistance in a *ry* and *ry*$^+$ background, the purine resistant strains were also crossed reciprocally to the wild type strain OR^R, and the numbers of F_1 survivors in the experimental and control vials were scored. The results are presented in Table 2b. The number of survivors in the experimental vials are not lower than those in the control vials. (In fact, the number of survivors in the control vials is sometimes lower, and this is probably because the control vials were a later brood, while the experimental vials were an earlier brood). From the results on Table 2b, it can be deduced that purine does not adversely affect survival of larvae in a *ry*$^+$/*ry* background.

4. Linkage Analyses

The Figures 1, 2 and 3, represent crosses that were designed to determine on which chromosome the gene(s) responsible for purine resistance (Pur^R) map. The genotype and number of F_2 survivors in the experimental and control vials are presented in Tables 3, 4 and 5. The following deductions can be made from the results: For P.R. 51 and P.R. 130, the Pur^R phenotype maps to chromosome 3. This is indicated by a lack of *Sb* flies in the experimental vials of cross #3 (Figure 3). It is further strengthened by substantial numbers of *Cy* and *Cy*$^+$ survivors on the purine in cross #2 (Figure 2), and by the lack of flies in cross #1 (Figure 1).

The Pur^R phenotype of P.R. 133 maps to chromosome 2. In view of the significant numbers of *Sb* and *Sb*$^+$ survivors in

Table 2 (b)

Cross *	Number of survivors in the experimental vials (+ Purine)	Number of survivors in the control vials (- Purine)
P.R. 51 x OR^R	255	257
P.R. 94 x OR^R	341	200
P.R. 130 x OR^R	330	169
P.R. 131 x OR^R	304	184
P.R. 133 x OR^R	302	125
P.R. 8-1 x OR^R	224	156
P.R. 8-11 x OR^R	224	151

* The purine resistant mutants were crossed to the wild type strain, OR^R. The numbers of survivors on medium with purine (0.2 ml of 0.2% purine) and without purine are shown.

Table 3 . X-Chromosome Linkage Tests for Purine Resistance.

Strain	Experimental vials: +P		Control vials: -P	
	Male	Female	Male	Female
P.R. 51	0	0	18	5
P.R. 94	0	0	14	5
P.R. 130	0	0	5	4
P.R. 131	0	0	103	57
P.R. 133	0	0	12	8
P.R. 8-1	0	0	26	7
P.R. 8-11	0	0	40	20

Males from the purine resistant strains were crossed to virgin females of the $\hat{X}\hat{X};cn;ry$ stock. The F_1's were allowed to develop on medium with purine (0.2 ml of .2% purine) and without (-P) purine. The numbers of males and females were scored in the vials.

Table 4 Second Chromosome Linkage Tests for Purine Resistance

	Experimental vials: +P		Control vials: -P	
Strain	*Cy*	Cy^+	*Cy*	Cy^+
*P.R. 51	55	161	69	127
P.R. 94	29	779	89	299
P.R. 130	41	115	43	82
P.R. 131	93	731	93	183
P.R. 133	1	521	32	144
P.R. 8-1	7	29	105	203
P.R. 8-11	118	322	41	185

* The purine resistant mutants were crossed to the *SM5 Cy/Sp;ry* stock as shown in Fig. 2 . The F_2's were allowed to develop on medium with purine (0.2 ml of 0.2% purine) and without (-P) purine. The *Cy* and the Cy^+ phenotypes were scored in the experimental and control vials.

Table 5 Third Chromosome Linkage Tests for Purine Resistance

	Experimental vials: +P		Control vials: -P	
Strain	*Sb*	Sb^+	*Sb*	Sb^+
*				
P.R. 51	0	395	111	105
P.R. 94	59	609	148	97
P.R. 130	0	295	102	73
P.R. 131	17	210	102	109
P.R. 133	159	305	128	112
P.R. 8-1	0	5	92	106
P.R. 8-11	37	215	148	133

*The purine resistant mutants were crossed to the *MKRS* stock as shown in Fig. 3 . The F_2's were allowed to develop on medium with purine (0.2 ml of 0.2%) and without purine (-P). The *Sb* and Sb^+ phenotypes were scored in the experimental and control vials.

cross #3, and lack of survivors in cross #1, the single *Cy* fly in cross #2 is assumed to be an escaper.

The results for the other four strains are not entirely clear, but some speculations can be made. For P.R. 94, the presence of both *Cy* and *Sb* survivors in the experimental vials (Tables 4 and 5), indicates that the purine resistance phenotype does not completely segregate from the *SM5* or *MKRS* chromosomes. It can therefore be speculated that P.R. 94 is a double mutant, carrying mutations on both chromosomes two and three, that render the strain resistant to purine. Similar speculations can be made for P.R. 131 and P.R. 8-11 as well. It is also possible that although P.R. 94, P.R. 131 and P.R. 8-11 may be double mutants, they only require one, or the other, mutation to express the Pur^R phenotype. Lack of flies in the experimental vials of cross #1 (Table 3) rules out X-chromosome linkage. Knowing that purine resistance maps to chromosome three in P.R. 51 and P.R. 130 and to chromosome two in P.R. 133, it is likely that complementation analyses involving crossing these mutants with each other, may shed some light on whether P.R. 94, P.R. 131, and P.R. 8-11 are double mutants.

Due to the low survival of P.R. 8-1 on purine (see Figure 4c), no conclusions can be drawn from the linkage analysis on this mutant. The few survivors in the experimental vials might not represent the actual segregation pattern.

5. Complementation Analysis: For the Purine Resistance Phenotype

To determine the number of loci involved in purine resistance

for the seven mutants isolated, a classical complementation analysis was performed. The purine resistant mutants were crossed with each other reciprocally. Two broods were made: purine was added to one brood (experimental vial) and not added to the other brood (control vial). The numbers of survivors in the vials were compared. The purine resistant mutant, #82 that was given to us by Victoria Finnerty, was also included in the complementation matrix. This mutant was characterized by Johnson and Friedman (1983), and purine resistance was found to map to the *Aprt* locus on chromosome 3. Therefore, inclusion of this mutant served to determine if our mutants were allelic to #82. Complementation (restoration of 'wild-type' phenotype) is indicated by a lack of F_1 survivors in the experimental vials. On the other hand, approximately equal numbers of survivors in the experimental and control vials, indicates non-complementation ('mutant' phenotype). In other words, if two allelic mutants are crossed, the F_1's survive on purine containing medium, as they are homozygous for the Pur^R gene.

The results are presented in Table 6. It can be seen that complementation certainly occurs in the combinations P.R. 133 x P.R. 51 and P.R. 133 x P.R. 130, and perhaps in P.R. 133 x P.R. 8-1 and P.R. 133 x #82. This indicates that the gene conferring purine resistance in P.R. 133 is different from those responsible for purine resistance in the mutants that it complements. The results of the combinations P.R. 133 x P.R. 94 and P.R. 133 x P.R. 131 do not clearly indicate complementation or non-complement-

Table 6 · Complementation Analyses: For the Purine Resistant Phenotype

The purine resistant mutants were crossed to each other reciprocally, and the flies were brooded twice. To one brood, 0.2 ml of 0.2% purine was added (+P), and no purine (-P) was added to the other brood. The numbers of F_1 survivors in the experimental and control vials were scored. If the number of flies in the experimental vials was approximately equal to that found in the control vials, it indicated non-complementation, and is represented by 'X' in the Table. Lack of (or very few) flies in the experimental vials, with substantial numbers in the control vials indicated complementation, and the corresponding numbers are indicated. (Mutant #82 served as a control, as described in the text).

Table 6

♂

♀	P.R. 51	P.R. 94	P.R. 130	♂82	P.R. 131	P.R. 8-11	P.R. 8-1	P.R. 133
P.R. 51		X	X	X	X	X	+P 12 -P>100	+P 0 -P > 100
P.R. 94	X		X	X	X	X	+P 35 -P>100	+P 54 -P > 100
P.R. 130	X	X		X	X	+P 8 -P 60	+P 0 -P 77	+P 0 -P 104
♂82	X	X	X		X	X	+P 22 -P>100	+P 6 -P > 100
P.R. 131	X	+P 35 -P > 100	X	X		X	+P 3 -P 87	+P 20 -P 77
P.R. 8-11	X	X	X	X	X		+P 20 -P>100	+P 20 -P > 100
P.R. 8-1	+P 26 -P > 100	+P 37 -P >100	+P 18 -P > 100	X	+P 25 -P > 100	+P 2 -P>100		+P 0 -P > 100
P.R. 133	+P 0 -P 69	+P 68 -P >100	+P 0 -P > 100	+P 0 -P > 100	+P 20 -P > 100	+P 0 -P 92	+P 0 -P>100	

ation. The unusual nature of P.R. 131 and P.R. 94, discussed above and elsewhere, may help to account for the results of the respective hybrids with P.R. 133. For example, the combination P.R. 133 x P.R. 94 may show partial non-complementation in the present experiment since P.R. 94 may share the P.R. 133 lesion.

In the crosses involving P.R. 8-1, the number of survivors in the experimental vials is considerably lower than those in the control vials, except in combination with P.R. 133, where no survivors are found in the experimental vials. It is possible that the apparently depressed number of survivors is because of the low survival of P.R. 8-1 on purine-containing medium. Thus, the results of the crosses involving P.R. 8-1 (except when crossed to P.R. 133) may be interpreted as indicating non-complementation. The reciprocals of the combination P.R. 8-1 x P.R. 133 both produce zero survivors in the experimental vials, and over 100 survivors in the control vials. This is indicative of complementation. Thus, the Pur^R phenotype in P.R. 8-1 probably maps to a different locus than P.R. 133.

The combinations P.R. 130 x P.R. 8-11, P.R. 8-1 x #82 and P.R. 133 x P.R. 8-11 behave differently in reciprocal crosses. These results need to be rechecked before definite conclusions can be drawn. Mutant #172 was not included in the complementation matrix as this mutant is not in a *ny* background.

From the data, it can be deduced that multiple loci may be responsible for conferring purine resistance. If one includes

the fact that a ry^+ genotype also confers substantial purine resistance, then taken together with the above mentioned results, it can be concluded that at least four loci are involved in purine resistance in *Drosophila*.

6. Biochemical Characterization

Johnson and Friedman (1981) proposed that purine resistant *Drosophila* are deficient for APRT activity. Their hypothesis for the mechanism of resistance, is that in APRT$^-$ strains, the administered purine cannot be converted to a toxic nucleotide, thus rendering the strains purine resistant. In view of this finding, the seven purine resistant mutants isolated in our laboratory were assayed for APRT activity. The assay is based on the synthesis of (^{14}C) AMP from (^{14}C) Adenine and PRPP by the APRT present in a fly homogenate. The compounds were separated by thin layer chromatography, and identified by running standards on the TLC plates. Representative results of the assays are shown as photographs of these autoradiograms in Figures 5a, 5b, 5c and 5d.

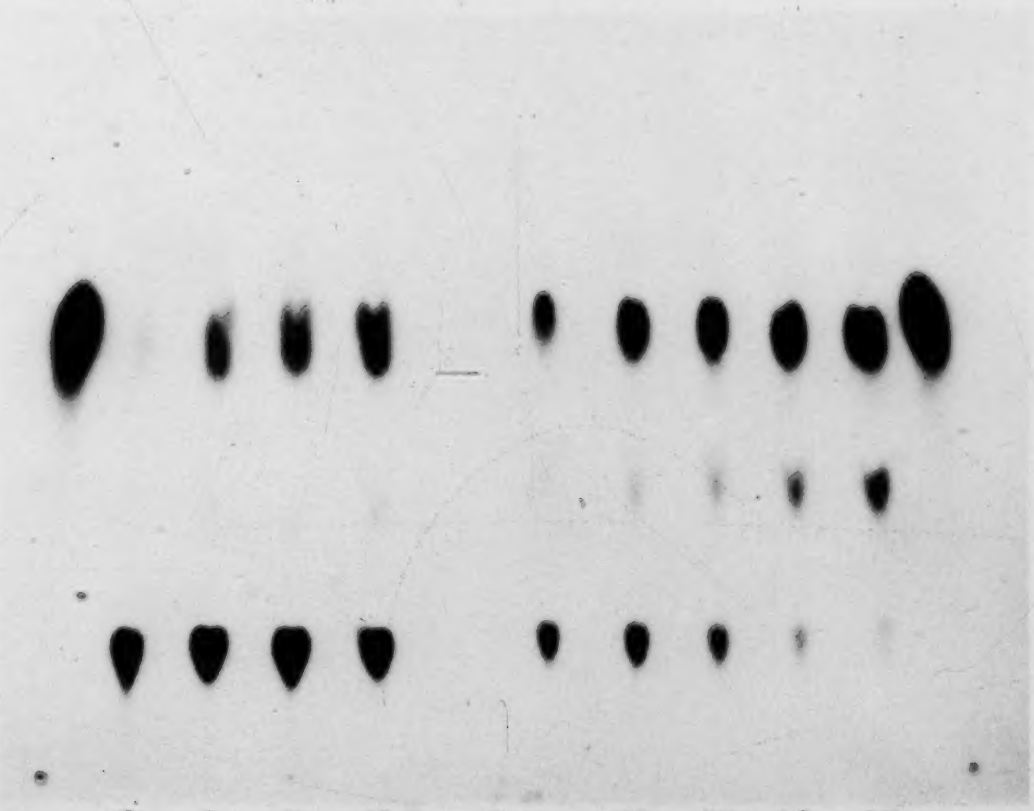
Figure 5a shows the synthesis of AMP in OR^R. (Radioactive AMP was run as a standard in the outer lanes.) As can be seen, the increasing intensity of the AMP spots is accompanied by the simultaneous decrease of the adenine spots showing that the synthesis of AMP from adenine and PRPP by the fly homogenate is apparently linear with time. The intermediate spots correspond to adenosine which is a breakdown product of AMP by AMP nucleo-

Figures 5a, 5b, 5c and 5d.

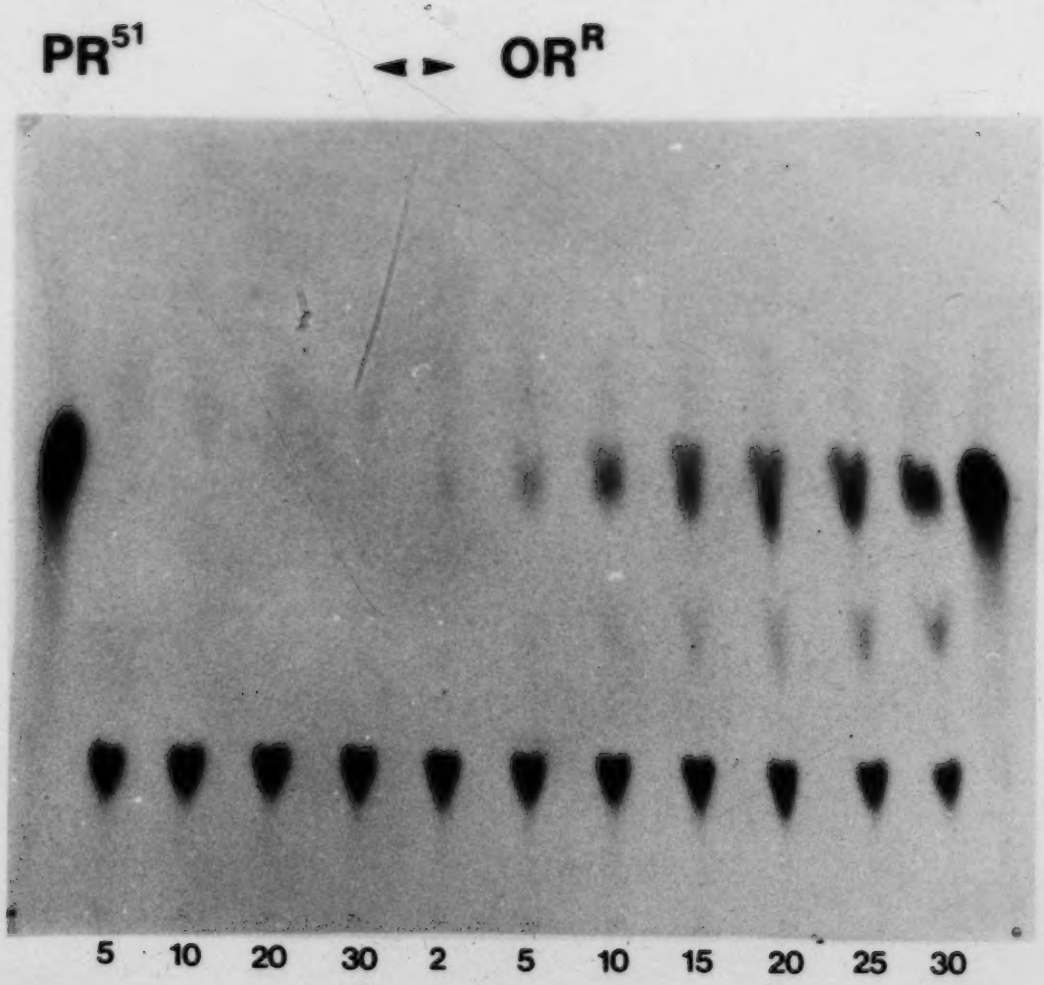
Assays for APRT Activity in OR^R, the Purine Resistant Mutants, and ry^{604}.

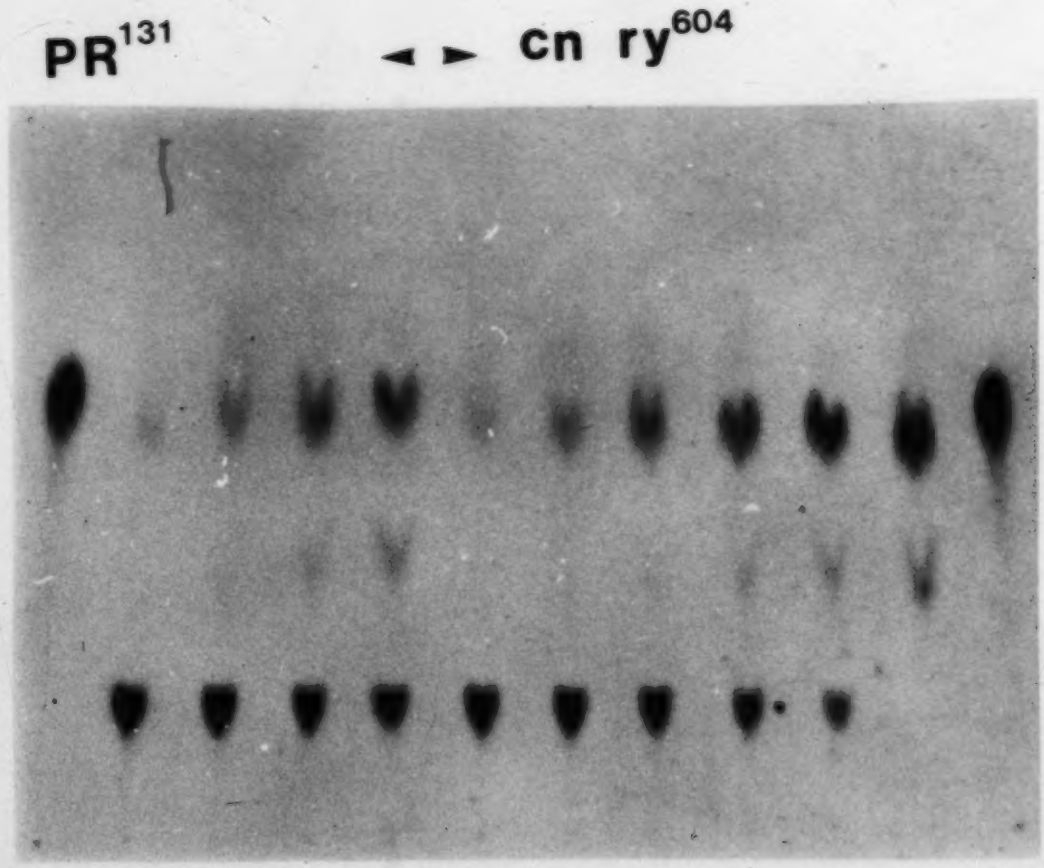
The different strains were assayed for APRT activity as described in Materials and Methods. Separate 2 µl samples of the assay mixtures were removed at the time points indicated, and applied to thin layer chromatography (TLC) plates. Radioactive AMP (2µl) was spotted on the outer lanes and served as a standard. Following development of the plates in 0.1 M LiCl, they were dried, and sprayed with Enhance. The plates were exposed to X-ray film for 2-3 days, after which the films were developed.

OR^{R} (WILD TYPE)



TIME IN MINUTES


PR51
◄►
ORR
5 10 20 30 2 5 10 15 20 25 30
TIME IN MINUTES


PR131
◄ ►
cn ry604
5
10
20
30
2
5
10
15
20
30
TIME IN MINUTES

PR[133]

5 10 20 30

TIME IN MINUTES

tidase (Johnson and Friedman, 1981). The radioactivity associated with the adenosine spots represents less than 10% of that in AMP, and was thus not included in the subsequent quantitation of APRT activity.

Figure 5b shows that no AMP was synthesized by any homogenates of P.R. 51, and this mutant strain is thus completely APRT$^-$. Two other mutant strains, P.R. 94 and P.R. 130, were also found to be APRT nulls. Figure 5c shows that P.R. 131 has some APRT activity. The *ry* strains from which the purine resistant strains were derived were also assayed. The synthesis of AMP by the homogenate from ry^{604} is also indicated in Figure 5c. The other purine resistant strains, P.R. 8-1, P.R. 8-11 and P.R. 133, were found to have considerable APRT activity. Figure 5d shows the synthesis of AMP in P.R. 133. The levels of APRT activity in each of these mutant strains in relation to wild-type is discussed below.

The radioactivity associated with the AMP spots in the mutants and controls was quantitated as described in Materials and Methods. APRT activity is expressed as cpm/µg protein. Figure 6 shows the kinetics of APRT activity in the wild type control, OR^R. As can be seen, the synthesis of AMP is linear up to 30' under the conditions employed. Figure 7 shows a typical set of results depicting the kinetics of APRT activity in the purine resistant strains, OR^R, and ry^{604}. The mutant strains P.R. 51, P.R. 94, and P.R. 130 were found to be APRT

Figure 6. Kinetics of APRT Activity in OR^R

The wild type strain OR^R was assayed for APRT activity as described in Materials and Methods. The enzyme activity, expressed as cpm/ug protein is plotted against time.

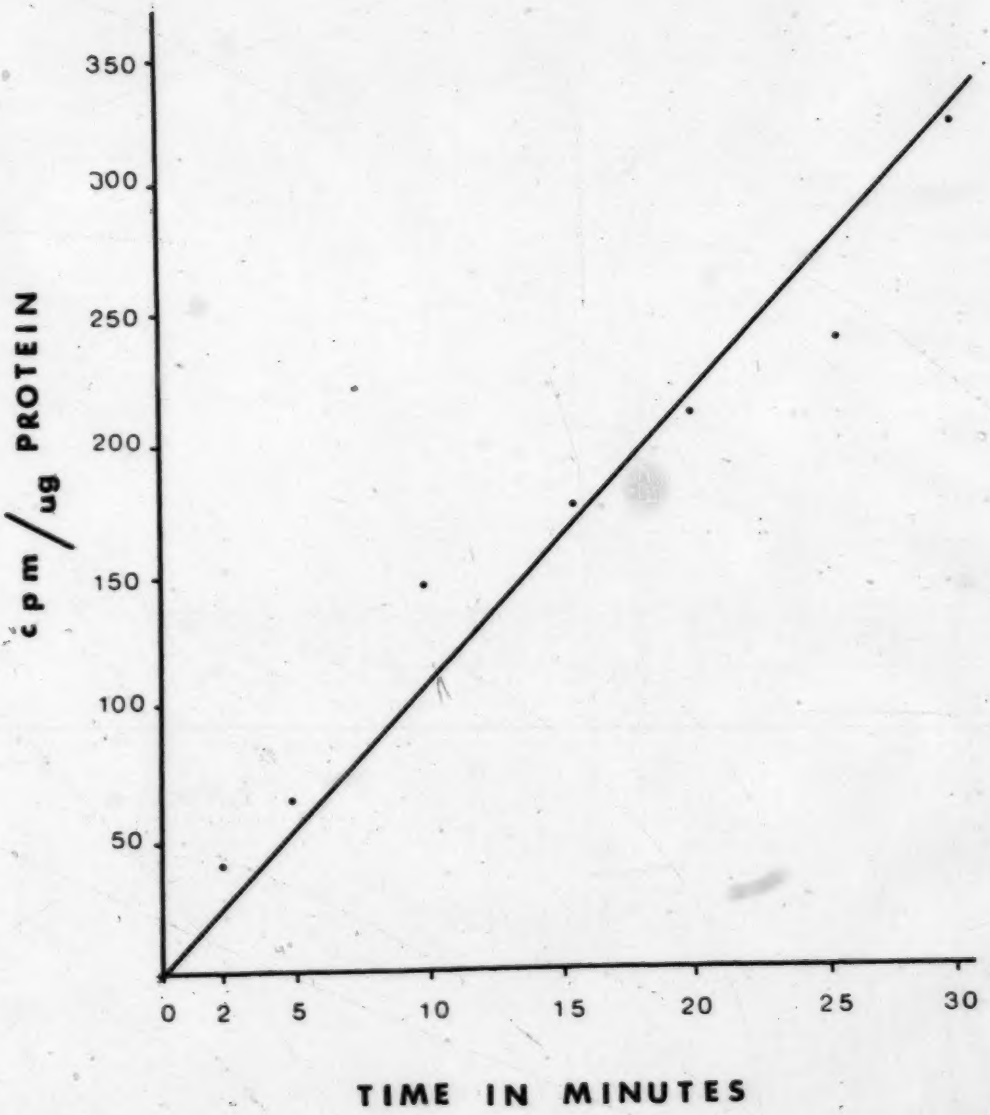
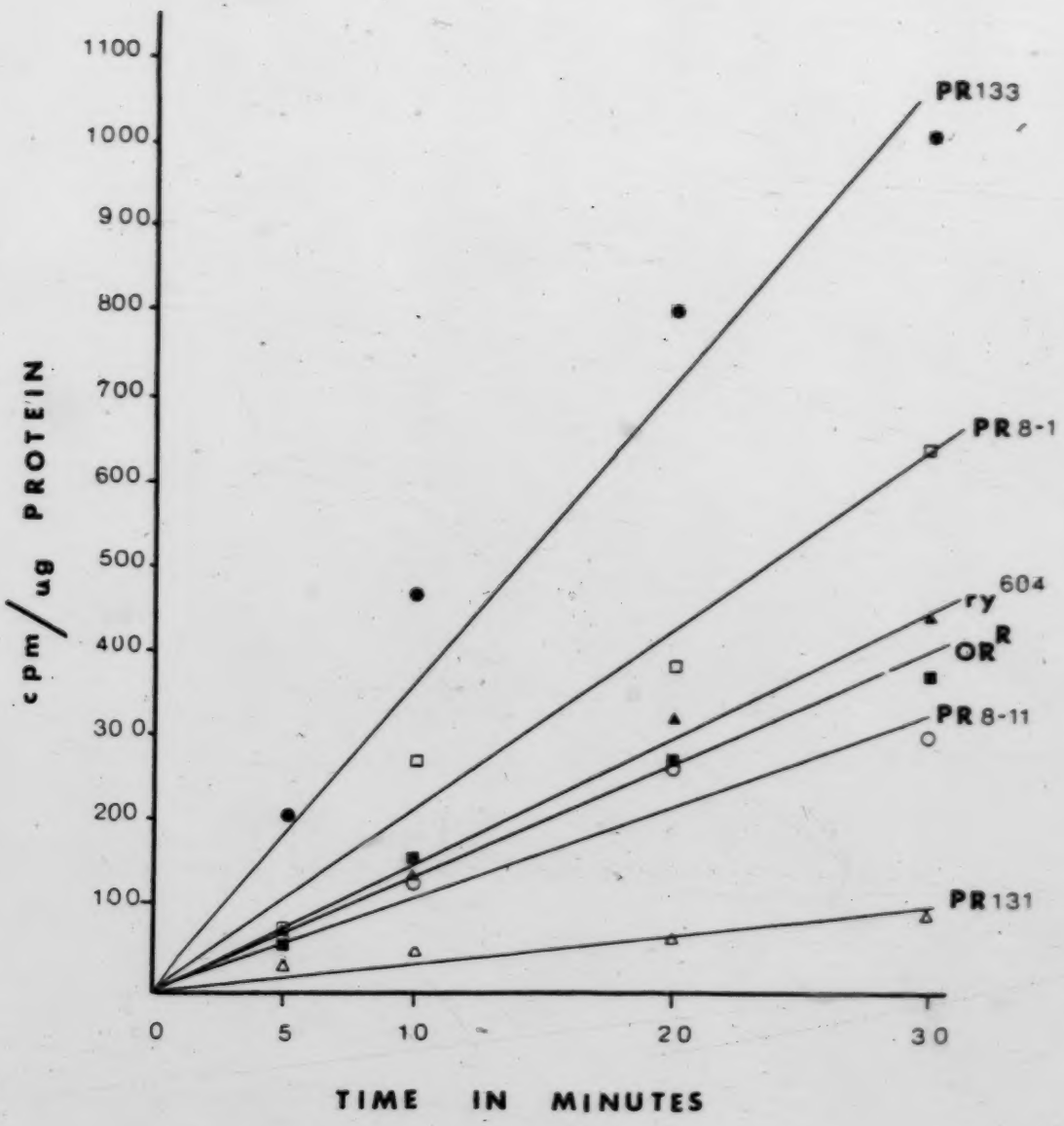

350
300
250
200
150
100
50
cpm / ug PROTEIN
0
2
5
10
15
20
25
30
TIME IN MINUTES

Figure 7 Kinetics of APRT Activity in the Purine Resistant Mutants, OR^R and ry^{604}. These data are from a representative kinetic determination.

The different strains were assayed for APRT activity as described in Materials and Methods. The enzyme activity (expressed as cpm/μg protein), at different time points, is plotted.


1100
1000
900
800
700
600
500
400
300
200
100
cpm / ug PROTEIN
PR133
PR 8-1
ry 604
OR R
PR 8-11
PR131
0
5
10
20
30
TIME IN MINUTES

nulls (see Table 7) and are thus not represented in the graph. As can be seen from Figure 7, P.R. 131 and P.R. 8-11 have activities lower than that of OR^R, while P.R. 8-1 and P.R. 133 have activities higher than wild-type, at least in this set of representative data. P.R. 133 consistantly has APRT activity substantially higher than ry^{604}, from which it was derived (see Table 7).

Table 7 presents the APRT activity in the different strains expressed as a percentage of the wild type OR^R. The counts at the 30 minute time point were used to make these comparisons, as that time point is within the linear part of the reaction (see Figure 6). These data differ from those depicted in Figure 7 in that they represent an average of all the determinations instead of just a representative set as in Figure 7.

The APRT activity of the *ry* strains, ry^{604} and ry^{8} is not significantly different from that of wild-type, as seen from Figure 7 and Table 7. This eliminates the possibility of P.R. 133 having higher than wild-type activity due to already elevated levels in the parent ry^{604} strain. The implications of P.R. 133 having an APRT activity higher than OR^R and ry^{604} are not clear at this point.

Since purine arrests the development of larvae of *ry* strains, it was of interest to determine the levels of APRT activity in the larvae and compare it with that in adults. Assays were done on larval homogenates from P.R. 51, P.R. 133 and OR^R. P.R. 51

Table 7 APRT Activities in Purine Resistant, *ry*, and Wild-Type Strains

Strain	% Wild Type Activity
OR^R	100
P.R. 51	0
P.R. 94	0
P.R. 130	0
P.R. 131	15
P.R. 133	216
P.R. 8-1	104
P.R. 8-11	44
#82	0
#172	0
cn ry^{604}	103
cn ry^{8}	96
Amh	86

The different strains were assayed for APRT activity as described in Materials and Methods. The activities at the 30 minute time point, expressed as a percentage of the activity in OR^R, are presented. The values indicated are an average of between 2-6 independent determinations.

and P.R. 133 were chosen, as they represent strains with no APRT activity and substantial APRT activity, respectively. It was found that the larvae of P.R. 51 lacks APRT activity, as in the case of adults. The larvae of P.R. 133 have APRT activity, like the adults (see Table 8). The fact that the larvae of P.R. 133 have APRT activity, eliminates the possibility of the strain being purine resistant because of a lack of APRT activity in the larval stage.

7. Complementation Analyses: For APRT Activity

The purine resistant strains were crossed to each other reciprocally, and the APRT activity of the F_1's assayed. Mutants #82 and #172 were also included in the complementation matrix as controls. These two mutants were found to be deficient for APRT activity (Johnson and Friedman, 1981, 1983), and purine resistance was found to map to the *Aprt* locus.

The purine resistant mutants were also crossed to OR^R flies, and the F_1's assayed for APRT activity (results included in Table 9b). The values obtained from this cross serve as a dosage control, as it is expected that in the absence of negative complementation, the contribution of the wild type in the F_1's would be approximately equivalent to one dose of the wild type activity.

Table 9a shows the results of the complementation analysis involving the APRT nulls. As can be seen, P.R. 51, P.R. 94 and P.R. 130 do not complement one another, nor #82 and #172. As

Table 8

APRT activity in larvae* expressed as % wild type activity

Strain	Number of determinations	APRT activity as % wild type
OR^R	3	100%
P.R. 51	3	0%
P.R. 133	3	79.3%

*2nd - 3rd instar larvae were homogenized in extraction buffer (150 mg/ml). The assay and quantitation of APRT activity is described in Materials and Methods. The average of three independent determinations, expressed as a percentage of wild type activity, is shown.

Tables 9a, 9b and 9c. Complementation Analyses: For APRT Activity

The purine resistant mutants were crossed to each other reciprocally, and the F_1's were assayed for APRT activity as described in Materials and Methods. Mutants #82 and #172 served as $APRT^-$ controls. The data for reciprocal crosses have been pooled. The APRT activity in the F_1's is expressed as a percentage of wild type activity. The values in the Tables represent an average of between two and seven determinations. The values for the individual determinations are presented in the Appendix.

Table 9a Complementation Results of APRT Nulls

	PR 51	PR 94	PR 130	#82	#172
PR 51	0	0	0	0	0
PR 94	0	0	0	0	0
PR 130	0	0	0	0	0
#82	0	0	0	0	0
#172	0	0	0	0	0

mentioned earlier #82 and #172 are mutant in the structural locus for APRT. Since P.R. 51, P.R. 94 and P.R. 130 do not complement these two mutants, it is logical to conclude that P.R. 51, P.R. 94 and P.R. 130 are allelic to #82 and #172 and therefore mutant at the *Aprt* locus.

Table 9b shows the complementation results of the APRT null mutants crossed to the mutant strains having APRT activity, as well as the levels of APRT activity in F_1 hybrids of OR^R and the purine resistant mutants. Given that the wild type allele contributes one dose equivalent to the activity in the F_1's, in crosses with the APRT nulls, it is expected that the percent activity in these F_1's will be approximately 50% compared to the homozygous wild type. As can be seen from Table 9b, with the exception of #82, the values obtained when OR^R is crossed to the nulls are fairly close to the expected value of 50%. The value of 68% obtained in the case of #82 is higher than expected and it is possible to speculate that in the heterodimer formed by the mutant polypeptide and the wild-type polypeptide, enzyme activity is partially restored (ie: the mutant polypeptide in this case contributes to the total activity). In the crosses involving OR^R and the strains having APRT activity, if the enzyme levels in the F_1 are determined by the additive effect of the contributing alleles, then the levels of enzyme activity in these F_1's can be predicted. For example, in the combination OR^R x P.R. 131, the F_1 might be expected to have 50% plus 7-10% ie. approximately 60%. However, a level of 84% suggests that

Table 9b APRT Activity Levels in the Indicated Hybrids

	P.R. 51	P.R. 94	P.R. 130	#82	#172	OR^R
P.R. 131	7	10	17	21	8	84
P.R. 133	62	53	69	91	70	109
P.R. 8-1	75	88	30	47	89	90
P.R. 8-11	52	16	43	49	18	76
OR^R	50	39	43	68	60	100

suggests that P.R. 131 is a leaky mutant at the *Aprt* locus, and that the heterodimer is active. The F_1's from P.R. 131 in combination with the APRT nulls would be expected to have approximately 7-10%. The values obtained with P.R. 51, P.R. 94 and #172 are in keeping with this expectation, while those obtained for P.R. 130 and #82 are higher. If the enzyme levels in the F_1's are strictly additive, the F_1's from combination P.R. 133 crossed with OR^R might be expected to have an enzyme activity approximately equal to 150%. However, the obtained value of 109% suggests that the Pur^R mutation in P.R. 133 is recessive in its effect on APRT activity. Knowing that purine resistance in P.R. 133 maps to a locus other than the *Aprt* locus, it is possible to speculate that the Pur^R mutation of P.R. 133 has a secondary effect on APRT activity. If this were true, one would expect that P.R. 133 in combination with the nulls, would produce a maximum of 50% activity in the F_1's. However, the results are confusing as only the values from P.R. 133 x P.R. 51 and P.R. 133 x P.R. 94 seem to be fairly close to the expected values.

Since P.R. 8-1 has approximately wild-type levels of APRT activity as a homozygote, its behaviour in combination with the nulls is expected to be similar to that of OR^R. With the exception of P.R. 8-1 x #82, the results are not in keeping with the expectation. The F_1's from the combination P.R. 8-1 and OR^R have an enzyme activity of 90%. This suggests that the Pur^R

mutation of P.R. 8-1 also has a recessive effect on APRT activity. If this were true, then the F_1's from P.R. 8-1 in combination with the APRT nulls, would be expected to have enzyme activity approximately equal to 50%. However, as can be seen in Table 9b, only the results of the cross P.R. 8-1 x #82 support this expectation. Thus, although the crosses of OR^R with P.R. 8-1 and P.R. 133 seem to indicate that the Pur^R mutations in these two strains are recessive in their effect on APRT activity, only some of the results support this idea. The Pur^R mutations of P.R. 8-1 and P.R. 133 are not allelic, as indicated from the results in Table 6.

The value of 76% obtained for the F_1's from P.R. 8-11 x OR^R is exactly as expected, but the results of P.R. 8-11 in combination with P.R. 51, P.R. 130 and #82 are higher than the expected value of approximately 25%.

Table 9c shows the levels of enzyme activity in the F_1's from crosses involving the purine resistant mutants having APRT activity. Assuming as before, that each parent contributes one dose equivalent and that the levels are additive, it can be seen from the results that some of the values fit the expectations. For example, as homozygotes, P.R. 131 and P.R. 8-11 have 15% and 44% of wild-type activity respectively (Table 7) and the F_1 from P.R. 131 and P.R. 8-11 has 30%, exactly as expected. However, the other results do not support the expectation. Further, if it assumed that the Pur^R mutations of P.R. 133 and 8-1 have a

Table 9C APRT activity levels in F_1's from $APRT^+$ purine resistant strains.

	P.R. 131	P.R. 133	P.R. 8-1	P.R. 8-11
P.R. 131	15			
P.R. 133	76	216		
P.R. 8-1	86	129	104	
P.R. 8-11	30	83	37	44

recessive effect on *Aprt*$^{+}$, then the value of 129% obtained for P.R. 8-1 x P.R. 133 is much higher than the expected value of 100%. Thus, some aspects of the results are confusing. As can be seen from the raw data of the results presented in the Appendix, the values for independent determinations vary considerably. Most often, the values obtained from using the first batch of [^{14}C] Adenine (ie. the value of 352 for the control and the corresponding values for the experimentals) are the ones that skew the results. It is possible that radiolysis of the radioactive adenine had occured, since this batch was at least ten years old.

However, the data allows one to draw the following conclusions: a comparison between Table 6 and Table 9a clearly shows, that at the level of purine resistance as well as APRT complementation, P.R. 51, P.R. 94 and P.R. 130 do not complement each other nor #82 and #172. Thus, this confirms that P.R. 51, P.R. 94 and P.R. 130 are mutant at the *Aprt* locus. For P.R. 51 and P.R. 130, this is further strengthened by the linkage analysis which indicates third chromosome linkage. Linkage analyses suggest that P.R. 94 is perhaps a double mutant, having a mutation on both chromosomes two and three. Since P.R. 94 is APRT^{-} and allelic to #82 and #172, it certainly carries a mutation on chromosome 3 (ie. at the *Aprt* locus). Further, since complementation analysis for purine resistance indicates that it may be allelic to P.R. 133 (Table 6), it strengthens the notion of

P.R. 94 being a double mutant. The mutant P.R. 133 is interesting and clearly different from the other mutants in several aspects.

8. Summary

From the results of the genetic and biochemical characterization, the following conclusions can be made. The linkage analyses show that in P.R. 51 and P.R. 130, the Pur^R gene maps to chromosome 3. They are APRT nulls, and in the complementation tests for APRT activity, they do not complement one another nor #82 and #172. In the complementation tests for purine resistance, they were both found to be allelic to #82. Since it is known that purine resistance in #82 and #172 maps to chromosome 3, and specifically to the *Aprt* locus, it can be concluded that in both P.R. 51 and P.R. 130, purine resistance is due to a lack of APRT, and thus in keeping with Johnson and Friedman's finding. The mutant P.R. 94 appears to be a fairly complex mutant. From the APRT assay, it was found to be a null mutant. From the complementation tests for purine resistance and APRT activity, it was found to be allelic to P.R. 51, P.R. 130 and #82. Yet, the linkage analyses data shows that the Pur^R gene does not clearly map to chromosome 3. Further, in the complementation tests for purine resistance, when crossed to P.R. 133, survivors were found in the experimental vials, although significantly less than in the control vials (see Table 6). This suggests that P.R. 94 could also be allelic to P.R. 133. But since P.R. 133 is not allelic to

P.R. 51, P.R. 130 and #82, it is possible to speculate that P.R. 94 is a double mutant. In other words, the purine resistant phenotype in P.R. 94 may be due to mutations at the *Aprt* locus, and at another locus, perhaps at the same locus as P.R. 133.

No definite conclusions can be drawn regarding P.R. 131, P.R. 8-1 and P.R. 8-11. The linkage data do not clearly indicate which chromosome the Pur^R phenotype maps to. The complementation patterns also appear to be quite random. However, it is possible that P.R. 131 is allelic to the nulls, but is a 'leaky' mutation. This would also help to explain its erratic behaviour in the complementation analyses.

P.R. 133 is a rather interesting mutant. Linkage analyses clearly show that the Pur^R phenotype maps to chromosome 2. In the complementation tests for purine resistance, complementation is indicated with all the other purine resistant mutants except P.R. 94 and perhaps P.R. 131. The fact that it complements P.R. 51, P.R. 130 and #82 show that it is not allelic to these mutants. This strengthens the notion that purine resistance in P.R. 133 is due to a reason other than being $APRT^-$. Further support of this idea comes from the fact that P.R. 133 has APRT activity substantially higher than wild type.

As an extension of the idea that purine resistance in *Drosophila* is due to a lack of APRT activity, Johnson and Friedman (1981) proposed that the degree of purine resistance is

inversely related to levels of APRT activity. Although this seems to be true for P.R. 8-1 and P.R. 133 when compared to the APRT nulls, (compare Table 7 with Figures 4a, 4b, and 4c) the correlation does not seem to exist in the case of P.R. 131 which is more resistant to purine at higher concentrations than the APRT null P.R. 51. Thus no general conclusions can be made regarding the correlations between levels of purine resistance and APRT activity.

From the results, it can be seen that purine resistance in *Drosophila* is fairly complex. The results from P.R. 133 clearly suggest that a locus other than *Aprt* is involved in purine resistance.

CONCLUSIONS

The finding that P.R. 133 is different from the other purine resistant mutants in *Drosophila* that are described in the literature leaves room for speculation regarding other possible bases of this phenotype in *Drosophila*. One possibility could be that P.R. 133 is a transport mutant, making purine inaccessible to its target organs, and thus rendering the mutants purine resistant. Two possible experiments could help to shed light on this hypothesis. Radiolabelled purine can be included in the culture medium of P.R. 133, as well as in the controls. (The controls could be a wild type strain and #82 or #172, which we know are not transport mutants). A comparison of the amount of label found in the homogenates from the experimental and control strains will help to discern if P.R. 133 is, indeed, a transport mutant. Alternatively, one could cross P.R. 133 to a purine auxotroph, and use a screen that will enable the selection of the double mutant that is both purine resistant and an auxotroph. The auxotroph requires exogenous purine to survive and is thus not impaired in its capacity of purine uptake and transport. If P.R. 133 is a transport mutant, then when crossed to an auxotroph, the double mutant (ie. purine resistant and auxotrophic) would be unable to survive the screen.

Another hypothesis is that P.R. 133 is mutant in a gene, which when mutated, is involved in detoxification of the administered purine. As mentioned in the Introduction, the mechanism of purine

resistance is often found to be associated with the loss of an enzyme activity that converts the purine or purine analogues to their ribotides. Since P.R. 133 has APRT activity (that directly converts the base to the nucleotide), it is possible to speculate that it lacks a nucleoside phosphokinase activity, that converts the nucleoside to the nucleotide. But this hypothesis presupposes that the administered purine is converted to the nucleoside. The nucleoside, however is primarily formed from the nucleotide by the action of a nucleoside phosphorylase (reviewed in El Kouni, 1977). If the 'toxic' nucleotide is already formed from the purine, it seems unlikely that the formation of the nucleotide *via* the nucleoside would be necessary to effect toxicity.

There have been reports indicating that the formation of the nucleotide from the administered purine bases or nucleosides is not always necessary to produce the toxic effects. Hershfield *et al.*, (1977) found that adenine and adenosine are toxic to human lymphoblasts that are deficient in APRT and adenosine kinase (AK). In other words, the inability of these mutants to form the nucleotide does not make them resistant to the base and nucleoside. Their results suggest that adenine and adenosine may be directly toxic to the cells. They suggest the existence of purine receptors that recognize adenine or adenosine (or both). In view of their finding, it is possible to hypothesize that perhaps P.R. 133 is unable to recognize the administered purine, due to defective purine receptors.

At the present time, it is not known which hypothesis is most likely to explain the behaviour of P.R. 133.

In addition to using radiolabelled purine to determine if P.R. 133 is a transport mutant, one could also do tracer studies with the purine to determine the other possible mechanisms of resistance. For example, by following the fate of the radiolabelled purine in normal, purine sensitive and purine resistant strains, it is possible to determine where in the pathway of purine utilization, each of the strains differs.

Tracer studies might also shed some light on why purine kills developing cultures of *ry* strains. This problem is curious in that strains that are XDH^- (ie. *ry* or *mal*) are very sensitive to purine. Yet the fact that we obtained purine resistant mutants that are XDH^-, and that some of the mutants characterized by Johnson and Friedman (1983) are in a wild type (ie. XDH^+) background, seems to indicate that other loci are also involved in purine resistance in *Drosophila*. Other intriguing aspects are, that purine is not a substrate for XDH, and that the response to purine is delayed, in that, development of *ry* cultures is arrested only at the third instar stage. However the arrest could be due to a cumulative effect of toxicity throughout development.

The characterization of the purine resistant mutants isolated in our laboratory shows that in at least two of the mutants (P.R. 51 and P.R. 130) purine resistance results from a deficiency of APRT, in agreement with the findings of Johnson and Friedman (1983). However, the behaviour of P.R. 133 clearly indicates that a locus other than

the *Aprt* locus is involved in purine resistance. Yet, these three mutants (P.R. 51, P.R. 130 and P.R. 133) were isolated from the same background; ry^{804}. Whether there is a connection between the *ry* and *Aprt* loci or their products is not clear at this point. The products of the two loci, XDH and APRT, both utilize purines for substrates, and are involved in the purine sensitivity of *Drosophila*. An analogous situation was found in cultured animal cells. Studies on drug resistance in mouse fibroblasts (Tomizawa and Aranow, 1960) revealed that cell lines resistant to 6-mercaptopurine (6-MP) are not only deficient in an enzyme system that converts 6-MP to its nucleotide, but also have a reduced capacity to convert hypoxanthine or inosine to inosinic acid. It was found that 6-MP exerted its toxic effect *via* the nucleotide, which somehow blocked DNA synthesis. Since it was found to be a potent inhibitor of cell growth, 6-MP has been used in the treatment of certain neoplastic disorders. It is hoped that the study of purine resistance in *Drosophila* will shed some light on the mechanism of action of the administered purine in all experimental organisms.

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APPENDIX

Raw Data of APRT Activity in Larvae

Strain	Experimental cpm/ug Protein	Control OR^R cpm/ug Protein	% OR^R Activity	Average
P.R. 51	833	9233	9	
P.R. 51	171	6375	2	4%
P.R. 51	105	3839	2	
P.R. 133	7426	9233	80	
P.R. 133	4460	6375	70	79.3%
P.R. 133	3396	3839	88	

* The counts at the 30 minute time point were used to make the comparisons

Raw Data of Complementation Analyses for APRT Activity

Cross	Experimental cpm/ug Protein *	Control OR^R cpm/ug Protein *	% OR^R Activity	Average
131 x 51	2329	22016	10	
131 x 51	1677	20497	8	
131 x 51	2767	13579	20	
131 x 51	0	23054	0	7%
51 x 131	790	13579	6	
51 x 131	0	352	0	
131 x 130	1412	5874	24	
131 x 130	5863	23054	25	17%
130 x 131	647	4698	13	
130 x 131	17	352	5	
131 x 133	155	352	44	
131 x 133	4864	4698	103	76%
133 x 131	18882	23054	82	
131 x 94	19	352	5	
131 x 94	499	4698	10	
94 x 131	8	23054	0	10%
94 x 131	6050	23054	26	
131 x 8-1	9289	22016	42	
131 x 8-1	9025	23054	40	
131 x 8-1	783	352	220	86%
8-1 x 131	513	352	145	
8-1 x 131	6963	23054	30	
8-1 x 131	8731	22016	40	
131 x 8-11	10197	23054	45	
131 x 8-11	132	352	37	30%
8-11 x 131	35	352	10	
8-11 x 131	6963	23054	30	
131 x 82	2439	22016	11	
131 x 82	4582	13579	33	
131 x 82	172	352	49	
82 x 131	1547	22016	7	21%
82 x 131	1521	13579	11	
82 x 131	1485	6356	23	
82 x 131	2794	23054	12	

Raw Data of Complementation Analyses for APRT Activity

Cross	Experimental cpm/ug Protein *	Control OR^R cpm/ug Protein *	% OR^R Activity	Average
131 x 172	0	352	0	
131 x 172	1580	6356	24	8%
172 x 131	0	352	0	
51 x 130	0	352	0	
130 x 51	0	23054	0	0%
51 x 133	271	352	77	
131 x 51	10950	23054	48	62%
51 x 94	0	352	0	
94 x 51	0	23054	0	0%
51 x 8-1	268	352	76	
8-1 x 51	16980	23054	74	75%
51 x 8-11	5327	7392	72	
51 x 8-11	2584	13579	19	52%
8-11 x 51	225	352	64	
51 x 82	0	23054	0	
51 x 82	0	352	0	
82 x 51	215	5874	3	0%
82 x 51	927	23054	4	
51 x 172	0	352	0	
172 x 51	0	352	0	0%
130 x 133	12020	23054	52	
133 x 130	5391	6356	85	69%
130 x 94	0	352	0	
94 x 130	0	352	0	0%
130 x 8-1	5687	13579	42	
130 x 8-1	29	352	8	30%
8-1 x 130	9148	23054	40	
130 x 8-11	59	352	17	
130 x 8-11	4469	13579	33	43%
8-11 x 130	276	352	78	

Raw Data of Complementation Analyses for APRT Activity

Cross	Experimental cpm/ug Protein *	Control OR^R cpm/ug Protein *	% OR^R Activity	Average
130 x 82	0	352	0	
82 x 130	385	20497	1	0%
130 x 172	0	352	0	
172 x 130	336	23054	1	0%
133 x 94	10632	23054	46	
133 x 94	5255	22016	24	
133 x 94	5290	13579	39	53%
94 x 133	16413	23054	71	
94 x 133	4021	4698	85	
133 x 8-1	10459	7392	141	
133 x 8-1	354	352	100	129%
133 x 8-1	12623	5874	214	
8-1 x 133	13569	23054	59	
133 x 8-11	13180	13579	97	
133 x 8-11	10231	23054	44	
133 x 8-11	109	352	31	83%
8-11 x 133	14991	23054	65	
8-11 x 133	6922	13579	51	
8-11 x 133	733	352	208	
133 x 82	8517	23054	37	
133 x 82	7277	6356	114	
133 x 82	11210	5874	190	91%
82 x 133	9162	23054	40	
82 x 133	15793	22016	72	
133 x 172	23459	23054	100	
133 x 172	18367	23054	80	70%
172 x 133	7174	23054	31	
94 x 8-1	6624	23054	29	
94 x 8-1	11210	5874	190	88%
8-1 x 94	10630	23054	46	
94 x 8-11	1720	4698	37	
94 x 8-11	28	352	8	
8-11 x 94	3309	22016	15	16%
8-11 x 94	476	23054	2	

Raw Data of Complementation Analyses for APRT Activity

Cross	Experimental cpm/ug Protein *	Control OR^R cpm/ug Protein *	% OR^R Activity	Average
94 x 82	0	352	0	
82 x 94	0	352	0	0%
94 x 172	0	23054	0	
172 x 94	0	23054	0	0%
8-1 x 8-11	2054	23054	9	
8-11 x 8-1	9081	13579	67	37%
8-11 x 8-1	8190	23054	35	
8-1 x 82	48	352	14	
8-1 x 82	7010	6356	110	47%
82 x 8-1	64	352	18	
8-1 x 172	314	352	89	
172 x 8-1	312	352	89	89%
8-1 x 82	122	352	35	
82 x 8-1	3768	20497	18	
82 x 8-1	6167	7392	83	49%
82 x 8-1	216	352	61	
8-11 x 172	4552	23054	20	
172 x 8-11	55	352	16	18%
82 x 172	1507	23054	7	
172 x 82	621	23054	3	0
172 x 82	562	23054	2	

* The counts at the 30 minute time point were used to make the comparisons.

The value 352 for the OR^R control and the corresponding values for the experimentals, are from the first batch of [^{14}C] Adenine used. All of the remaining values are from the second batch of [^{14}C] Adenine.

Raw Data of APRT Activity in the F_1 of OR^R X P.R. Mutants

Cross	Experimental cpm/ug Protein *	Control OR^R cpm/ug Protein *	% OR^R Activity	Average
131 x OR^R_R	6407	10106	63	
131 x OR^R_R	4394	5253	83	
131 x OR^R_R	4974	3839	129	84%
131 x OR^R	4459	7392	60	
51 x OR^R_R	5862	10106	58	
51 x OR^R	1620	3839	42	50%
130 x OR^R_R	2754	10106	27	
130 x OR^R	2303	3839	59	43%
133 x OR^R_R	6739	10106	66	
133 x OR^R_R	14796	21245	70	
133 x OR^R_R	10078	5253	191	109%
133 x OR^R	7911	7392	27	
94 x OR^R_R	5011	10106	50	
94 x OR^R	5640	21245	27	39%
8-1 x OR^R_R	13333	10106	131	
8-1 x OR^R_R	12612	21245	59	
8-1 x OR^R_R	7393	7206	102	90%
8-1 x OR^R_R	2515	5253	47	
8-1 x OR^R	8245	7392	111	
8-11 x OR^R_R	8763	10106	86	
8-11 x OR^R_R	5750	21245	27	76%
8-11 x OR^R	6023	5253	114	
82 x OR^R_R	9760	10106	96	
82 x OR^R_R	5090	21245	24	
82 x OR^R_R	3171	7206	44	68%
82 x OR^R	5651	5253	107	
172 x OR^R_R	4717	7206	65	
172 x OR^R	5543	10106	54	60%

*The counts at the 30 minute time point were used to make the comparisons.